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(54) Title: MONOCLONAL ANTIBODIES TO LEUKOCYTE ADHESION MOLECULE-1

(57) Abstract

A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-1, -2, -4, -5, -6, -7, -8, -9, -10, -11, -14 or -15; the hybridoma cell which produces such monoclonal antibody; and methods of using such monoclonal antibody.

MONOCLONAL ANTIBODIES TO LEUKOCYTE ADHESION MOLECULE-1

This application is a continuation-in-part of Tedder et al., U.S.S.N. 07/720,602, filed June 25, 1991, and Tedder, U.S.S.N. 07/700,773, filed May 15, 1991, both of which are continuations-in-part of Tedder, U.S.S.N. 07/313,109, filed February 21, 1989, each of which is herein incorporated by reference.

Part of the work leading to this invention was made with funds provided under grants AI 26872 and CA 34183 by the United States Government, which has certain rights in this invention.

This invention relates to human leukocyte-associated cell surface proteins, and monoclonal antibodies specific for such proteins.

Background of the Invention

Genes exclusively expressed by one cell lineage, but not by others, often define the function of that cell population. The generation of genes by the assembly of functionally independent domains has occurred frequently as new genes have evolved to encode proteins with new functions. An inducible endothelial-leukocyte adhesion molecule (ELAM-1), having several functionally independent domains, is expressed on the surface of cytokine-treated endothelial cells. This molecule is thought to be responsible for the accumulation of blood leukocytes at sites of inflammation by mediating the adhesion of cells to the vascular lining (Bevilacqua et al., Proc. Natl. Acad. Sci. USA 84:9238 (1987)). A granule membrane protein found

done by any method or combination of methods for such determinations generally known to immunologists, including the following:

1. A recombinant gene encoding a chimeric protein composed of one of the LAM-1 functional domains (e.g., the lectin domain) fused to the other domains (e.g., the EGF-like and SCR domains) from another selectin could be used to assay for binding by the stated anti-LAM1 mAb and by the antibody to be tested. If one binds to the chimeric protein while the other does not, the two mAbs have different epitopes.

2. An antibody binding to its epitope can block the subsequent binding of a different antibody recognizing the same or a physically proximal epitope. Thus, if a first antibody bound to its epitope blocks the binding of a second antibody, the two are likely to recognize the same epitope.

3. LAM-1 functions are carried out by specific regions of the molecule, which are composed of individual epitopes. Therefore, an epitope involved in ligand binding, for example, can be identified by the ability of an antibody bound to that epitope to inhibit a given LAM-1 function, such as the binding of LAM-1 to PPME, fucoidin, HEV, or activated endothelial cells. Also, the binding of an antibody to its epitope may completely or only partially inhibit the given LAM-1 function, depending on the proximity of the epitope to the ligand binding site of the protein, so that the epitopes of two different antibodies may be distinguished on the basis of the difference in degree of inhibition by the two antibodies.

4. Some epitopes of LAM-1 may also be involved in the regulation of function of the molecule, such that antibody binding to those epitopes may augment a given LAM-1 function. For example, antibody binding to the LAM1-1 or

expressing LAM-1; contacting the cells with a reagent which includes the mAb; and determining which cells form an immune complex with the reagent. This reagent may be the mAb alone (e.g., in the form of a purified preparation or unfractionated ascites fluid), or may be the mAb conjugated with a detectable label (such as a detectable enzyme, fluorophore, or radioactive moiety). Determination of which cells form an immune complex with the reagent may be accomplished by any of a variety of standard immunological techniques, including immunoprecipitation, indirect or direct immunofluorescence with flow cytometry analysis, or immunosorbent assays.

In addition, the mAb of the invention may be used in a method of isolating cells expressing LAM-1, which method includes the steps of providing a sample of cells, at least some of which are suspected of expressing LAM-1; contacting the cells with the mAb or a reagent including the mAb; and separating those cells which have formed an immune complex with the mAb, from those cells which have not. This separation may be performed by any of a variety of standard immunological techniques, including fluorescence-based cell sorting, magnetic bead-based separation protocols, and the use of solid phase bound antibody.

The mAb of the invention is also useful in a diagnostic assay for detecting leukocyte activation in an animal, which activation may be attributable, for example, to inflammation, an autoimmune response, or rejection of an organ or tissue transplant. This method is accomplished by obtaining a fluid sample from an animal (preferably a human), which sample may be, for example, blood, serum, plasma, saliva, tears, cerebral spinal fluid, or urine; contacting the sample with the mAb of the invention; and detecting (by standard immunological techniques) formation

recipient, which method includes administering to the patient a therapeutic agent including a therapeutic amount of the LAM-1 protein or a domain thereof, or of an antagonist to the LAM-1 protein or domain thereof, or of a fusion protein including the LAM-1 protein or a domain thereof covalently bonded to an immunoglobulin heavy chain constant region. The therapeutic agent is administered in a pharmaceutically acceptable carrier substance.

In another aspect, the invention features using the LAM-1 protein or domain thereof to identify a ligand that binds to the protein or to a molecule that is specifically associated with the protein, or fragment thereof, to generate a functional molecule. Ligands so identified can also be used in the methods of the invention described above.

As used herein the term "antagonist to LAM-1" includes any agent which interacts with LAM-1 and interferes with its function, e.g., antibody reactive with LAM-1 or any ligand which binds to LAM-1. The term "identify" is intended to include other activities that require identification of an entity, such as isolation or purification. The term "essentially purified" refers to a protein or nucleic acid sequence that has been separated or isolated from the environment in which it naturally occurs.

Leukocyte-associated cell surface protein LAM-1 plays an important role in leukocyte-endothelial cell interactions, especially selective cell trafficking to sites of inflammation. The LAM-1 protein or domains thereof, or other molecules that interfere with leukocyte adhesion and function, can be used therapeutically to inhibit the inflammatory response and to treat such conditions as tissue damage and metastasis of cancer cells.

intensity of staining with each mAb was rated on a (+) to (++++) scale as shown on a three decade log scale. The background staining obtained using an unreactive isotype-matched mAb was less than 5% as shown by the shaded histograms in the first panels.

Fig. 10 depicts modulation of PPME binding by anti-LAM-1 mAb. Flow cytometry analysis of PPME-FITC binding to human blood mononuclear cells after treatment of the cells with medium or anti-LAM-1 mAb 20 min prior to PPME-FITC staining. PPME staining is completely inhibited by 5 mM EDTA (shaded panel), indicating that all of the PPME binding by PBMC is due to LAM-1. This is confirmed by the observation that inhibition by LAM1-3 also reduces the level of PPME staining to background.

Fig. 11 depicts evolutionary conservation of the LAM1-3 epitope. Blood mononuclear cells from a human, cow, rhesus monkey, dog, cat and rabbit were examined in indirect immunofluorescence assays for expression of the LAM-1 epitope identified by the anti-LAM1-3 mAb (dark line). The fluorescence histogram of cells treated with an unreactive murine IgG_1 mAb are also shown (thin line). Cells were examined by flow cytometry analysis and relative fluorescence intensity of staining is shown on four or three decade scales as indicated. Cell samples were examined at different times with different flow cytometer settings so that individual histograms are not directly comparable.

Fig. 12 depicts conservation of the PPME binding receptor by human, tamarin, dog, and rabbit blood mononuclear cells. Cells were incubated with PPME-FITC (3 μg/ml) in medium, medium containing saturating concentrations of the anti-LAM1-3 or anti-LAM1-4 mAb or medium containing 5 mM EDTA. Relative fluorescence

al., Eur. J. Immunol. 20:1351, 1990) and that of neutrophil LAM-1 is 90-100,000 (Kansas et al., J. Immunol. 142:3058, 1989). This presumably results from different patterns of post-translational processing as occurs with many molecules expressed by both cell lineages. LAM-1 affinity for ligand is transiently increased following leukocyte activation with lineage-specific agents, which may partially explain the differences in migration between neutrophils and lymphocytes (Spertini et al., Nature 349:691, 1991). Among lymphocytes, LAM-1 is expressed by the majority of circulating T and B cells, is lost following mitogen stimulation, but is found on some antigen-specific memory T cells (Tedder et al., Eur. J. Immunol. 20:1351, 1990; Kanof et al., J. Immunol. 140:3701, 1988). LAM-1 is a member of the selectin family of cellular adhesion/homing receptors, which play important roles in leukocyte-endothelial cell interactions, especially selective cell trafficking to sites of inflammation. Like other members of this family, LAM-1 contains an amino-terminal lectin-like domain, followed by an epidermal growth factor (EGF)-like domain and short consensus repeat units (SCR) similar to those found in C3/C4 binding proteins. The lectin domain appears to interact with specific glycoconjugates expressed on high endothelial venules (HEV) of peripheral lymph nodes. This interaction is calcium dependent and can be inhibited by mannose 6-phosphate or mannose 6-phosphate rich polysaccharides, such as the phosphomannan monoester fragment, PPME. PPME is particularly useful as a soluble ligand since it can be labelled and used to assess LAM-1 binding activity without the influence of other adhesion molecules involved in leukocyte binding (Spertini et al., Nature 349691, 1991; Spertini et al., Leukemia (in press) 1991).

occurred. One of the 261 RAJI+ HSB2- cDNA clones isolated, B125, contained a 1.90 kb cDNA insert that hybridized with a 2.4 kb RNA species found in several B cell lines (Tedder et al., supra). However, B125 did not hybridize with any of the other RAJI+ RSB2- clones or with mRNA from several T cell lines. The B125 cDNA clone was characterized by restriction mapping and nucleotide sequence determination. A near-full-length 2.3 kb cDNA that hybridized with B125 was isolated, sequenced, and termed pLAM-1.

As shown in Fig. 1A, a restriction map was constructed by the standard single, double or triple digestions of pLAM-1. The coding region is shown in black. Arrows indicate the direction and extent of nucleotide sequence determination and the open circles indicate 5'-end labeling. In Fig. 1B, a schematic model of the structure of the LAM-1 mRNA is shown. Thin lines indicate 5' and 3' untranslated sequences (UT), while the thick bar indicates the translated region. The boxes represent the lectin-like and epidermal growth factor (EGF)-like domains and the two short consensus repeat (SCR) units. The open box indicates the transmembrane (TM) region.

pLAM-1 contains a 1,181 bp open reading frame that could encode a protein of 372 amino acids, as shown in Fig. 2. The numbers shown above the amino acid sequence designate amino acid residue positions. The numbers to the right indicate nucleotide residue positions. Amino acids are designated by the single-letter code, and * indicates the termination codon. The boxed sequences identify possible N-linked glycosylation sites. Hydrophobic regions that may identify signal and transmembrane peptides are underlined. The amino acid sequence of LAM-1 indicates a structure typical of a membrane glycoprotein. The mature LAM-1 protein has an extracellular region of about 294 amino

263:9557 (1988)). The lectin domain includes amino acid residues 42-170 given in Fig. 2.

The next domain of 36 amino acids, at residues 171-206 shown in Fig. 2, is homologous (36-39%) with epidermal growth factor (EGF) (Gregory, Nature 257:325 (1975)) and the EGF-like repeat units found in Factor IX (Yoshitake et al., Biochem. 25:3736 (1985)) and fibroblast proteoglycan core protein (Krusius et al., supra) (Fig. 3B).

Immediately following these domains are two tandem domains of 62 amino acids each, shown as residues 207-269 and 270-331 of Fig. 2, that are homologous with the short consensus repeat unit (SCR) that comprises the IL-2 receptor (Leonard et al., Nature 311:626 (1984)), Factor XIII (Ichinose et al., Biochem. 25:4633 (1986)) and many C3/C4 binding proteins (Klickstein et al., J. Exp. Med. 165:1095 (1987); and Morley et al., EMBO J. 3:153 (1984)). In contrast with all of the previously described SCR that contain four conserved Cys residues, each of these two SCR possesses six Cys residues. The four conserved Cys residues found in all SCR are indicated in Fig. 3C by (*); the two additional conserved Cys residues found in LAM-1 are indicated by (+). Of the multiple SCR present in each of these proteins, the SCR with the highest homology to LAM-1 is diagrammed (Fig. 3C). A 15 amino acid spacer follows the SCR units, preceding the transmembrane domain.

The expression of LAM-1 mRNA by cell lines of lymphoid and non-lymphoid origin was examined. Northern blot analysis revealed that LAM-1 cDNA hybridized strongly to a 2.6 kb RNA species and weakly to a 1.7 kb RNA species in poly(A)+ RNA isolated from the B cell lines Raji, SB, Laz-509, and GK-5. However, RNA isolated from two pre-B cell lines (Nalm-6, PB-697), three B cell lines (Namalwa, Daudi, BJAB), five T cell lines (CEM, Hut-78, HSB-2, Molt-

Cell samples and immunofluorescence analysis. Mononuclear cells were isolated from human, rhesus monkey (*Macaca mulatta*), cotton-topped tamarin (*Saguinus oedipus*), dog (*Canis familiaris*), cat (*Felis catus*), sheep (*Ovis aries*) or rabbit (*Oryctolagus cuniculus*) blood by Ficoll-Hypaque density gradient centrifugation. Neutrophils were isolated from blood samples at 20°C by centrifugation for 20 min at 1000 x g on a cushion of Mono-Poly Resolving Medium (Flow Laboratories, McLean, VA) followed by lysis of the red blood cells with ice-cold hypotonic 0.2% (w/v) NaCl solution. Cells were obtained by protocols approved by the Human Protection Committee and the Animal Care and Use Committee of Dana-Farber Cancer Institute. Cells were kept at 4°C and examined immediately after isolation.

Indirect immunofluorescence analysis was carried out after washing the cells three times. The cells were then incubated for 20 min on ice with each mAb as ascites fluid diluted to the optimal concentration for immunostaining. After washing, the cells were treated for 20 min at 4°C with FITC-conjugated goat anti-mouse Ig antibodies (Tago, Burlingame, CA). Single color immuno-fluorescence analysis was performed on an Epics Profile flow cytometer (Coulter Electronics, Hialeah, FL) or a FACStar (Becton Dickinson, Mountain View, CA). Ten thousand cells were analyzed in each instance.

Tissue sections were isolated from thymus and mesenteric lymph nodes of rabbit, pig (*Sus scrofa*), goat (*Capra hircus*), rat (*Rattus norvegicus*), guinea pig (*Cavia porcellus*) and chicken (*Gallus domesticus*), and from rabbit appendix. These frozen sections were stained with a given anti-LAM-1 mAb at optimal concentrations, with subsequent development using immunohistological procedures as described (Mackay et al., J. Exp. Med. 167:1755, 1988).

LAM-1 mAbs as ascites fluid diluted at 1:100 in RPMI 1640 containing 5% fetal calf serum. The anti-LAM1-3 and -4 mAb could be used at considerable dilution (1:5000) without a decrease in their ability to inhibit HEV binding. The cells, in a final volume of 100 µl, were then incubated under rotation (64 rpm) for 30 min at 4°C on four 12 µm frozen rat peripheral lymph node sections. After fixation overnight in PBS with 1% (w/v) glutaraldehyde, the number of HEVs per tissue section was determined and the number of lymphocytes adherent to HEV was quantitated.

Antibody cross-blocking experiments. Lymphocytes (5×10^5) were first incubated with 10-fold saturating concentrations of one anti-LAM-1 mAb as diluted ascites fluid for 20 min on ice followed by the addition of optimal concentrations of the second fluorochrome-labelled anti-LAM-1 mAb to be used for direct immunofluorescence analysis. After 20 min of further incubation, the cells were washed and mAb binding assessed immediately by flow cytometry, as described (Tedder et al., J. Immunol. 144:532, 1990). Optimal concentrations for each antibody were determined by indirect immunofluorescence analysis.

Domain mapping of epitopes defined by anti-LAM-1 mAb. As described in detail elsewhere (Kansas et al., J. Cell Biol. (in press) 1991), various domains of CD62 were substituted for those of LAM-1 to create chimeric selectins containing specific domains of LAM-1 with the remainder of the protein as CD62. In particular, cDNAs encoding fusion proteins containing the lectin domain, the lectin plus EGF domains, or the lectin, EGF plus SCR domains of LAM-1, with the remainder as CD62, were created and subcloned into the Ap^rM8 expression vector (provided by Dr. Lloyd Klickstein, Center for Blood Research, Boston, MA). These cDNAs were

staining with the anti-LAM-1 mAb ranged from low staining (+) to bright staining (++++) (Fig. 9, Table 7). No mAb were detected that stained lymphocytes or neutrophils preferentially. The level of staining observed was characteristic of each mAb, and was not due to low or insufficient mAb concentrations, because mAb levels greater than saturation generated identical results; or to isotype differences, because 14 of the 17 antibodies were of the same Ig subclass (i.e. IgG_1).

Inhibition of Lymphocyte binding to HEV is blocked by some anti-LAM-1 mAb. The anti-LAM-1 mAb were tested for their ability to inhibit lymphocyte binding to HEV of rat peripheral lymph node sections in the *in vitro* frozen section assay (Stamper et al., J. Exp. Med. 144:828, 1976). The anti-LAM1-3, anti-LAM1-4 and anti-LAM1-6 mAb inhibited lymphocyte binding by 85 to 90% (Table 8). In contrast to our previous report (Tedder et al., J. Immunol. 144:532, 1990), the anti-LAM1-1 and anti-LAM1-2 mAb consistently inhibited binding to an intermediate degree (~65% and ~45%, respectively). Our previous inability to detect this inhibition may be secondary to the previous use of human lymph nodes as a source of HEV; the likely involvement of LFA-1 in binding to (presumably inflamed) human lymph nodes (Stamper et al., J. Exp. Med. 144:828, 1976), may have obscured the true effects of these mAb. The remaining 12 anti-LAM-1 mAb had little or no effect on lymphocyte binding to HEV under the conditions examined (Table 7). This lack of effect did not result from the presence of insufficient mAb for inhibition, since each mAb was used at a concentration 2 to 10 fold higher than that required for saturation, as determined by immunofluorescence staining.

LAM-1 can inhibit PPME binding while mAb binding to different epitopes can upregulate binding.

Epitope localization of the anti-LAM-1 mAb binding sites. The spatial relationships between the different mAb binding epitopes were examined by mAb cross-blocking analysis (Table 9). Anti-LAM1-3 binding inhibited the subsequent binding of anti-LAM1-4, anti-TQ1 and anti-LAM1-2, but not anti-LAM1 mAb. Leu-8 binding was partially or totally blocked by most of the other mAb with the exception of anti-TQ1, anti-LAM1-2, -6 and -14. Taken together, this suggests that the anti-LAM1-3 and -4 epitopes are close to, but distinct from, the Leu-8 epitope, and farther from the epitopes defined by the other mAb. Anti-LAM1-7 and -8 partially, and -9, -10, -11, -12 and -15, totally, inhibited anti-LAM1-1, -5 and Leu-8 binding, indicating that these epitopes are clustered closely together. Anti-LAM1-6 appeared unique in this analysis, and therefore defines a spatially distant determinant. Finally, anti-LAM1-14 binding partially inhibited binding of anti-LAM1-1 only. Therefore, while most mAb-defined epitopes appeared to be closely associated within regions involved in ligand binding, distinct mAb-binding epitopes could be distinguished.

Domain mapping of epitopes identified by anti-LAM-1 mab. To identify the structural domains that contain epitopes defined by the anti-LAM-1 mAb, COS cells were transfected with cDNAs encoding chimeric (LAM-1/CD62) selectins and stained with anti-LAM-1 mAb (Table 10). With the exception of anti-LAM-1, -5, -14 and -15, each mAb bound the fusion protein containing only the lectin domain of LAM-1, and hence recognized the lectin domain. In combination with the mAb crossblocking analysis described above, this indicates that multiple antigenic sites are present within

detected (Table 11). The exceptions to this were the epitopes identified by the anti-LAM1-8 and -9 mAb and the epitopes identified by the anti-LAM1-10, -11 and -12 mAb, which recognized LAM-1 from the same species.

The finding that human and mouse lymphocytes bind PPME was extended to the study of tamarin, dog and rabbit LAM-1$^+$ lymphocytes (Fig. 12). PPME binding to lymphocytes from each of these species was completely inhibited by 5 mM EDTA. In addition, preincubation of cells from each of these three species with anti-LAM1-3 or -4 mAb diminished, albeit incompletely, PPME-FITC binding (Fig. 12). Finally, in two experiments, treatment of rhesus monkey blood mononuclear cells with anti-LAM1-3 inhibited 53% and 88% of lymphocyte binding to HEV, while treatment of dog mononuclear cells with anti-LAM1-3 inhibited 52% of the HEV binding. Thus, the functional and serologic properties of LAM-1 appear to be well conserved in diverse and phylogenetically disparate groups of mammals.

DISCUSSION

In this study, the LAM-1 epitopes which mediate or regulate HEV- and PPME- binding were characterized using a large panel of newly developed mAb (Table 7). In addition, the physical proximites of the functional regions defined by these mAb were identified, indicating that LAM-1 can be divided into a number of overlapping regions associated with distinct functional properties. Each mAb reacted with leukocytes with characteristic levels of immunofluorescence staining (Fig. 9, Table 7), suggesting that there may be heterogeneity in expression of LAM-1 epitopes. Thus, many of the anti-LAM-1 mAb may resemble the FMC46 mAb that identifies a cell protrusion-associated epitope of LAM-1. These mAb were also used to examine structural differences

to bind both HEV and PPME. The DREG-56 mAb also fits into this functionally defined group of mAb (Kishimoto et al., Proc. Natl. Acad. Sci. USA 87:2244, 1990). The anti-LAM1-3 and -4 mAb bind the lectin domain of LAM-1 only (Table 10), and each completely blocked binding of the other (Table 9). The anti-LAM1-3 and anti-LAM1-4 mAb also completely block neutrophil binding of PPME. Two additional mAb, anti-LAM1-2 and TQ-1, also blocked PPME binding (Fig. 10). However, these mAb were less efficient at blocking lymphocyte binding to HEV (Table 8) suggesting that they may be more distant from the ligand binding site than the LAM1-3 and -4 epitopes. These mAb collectively define one functional group of LAM-1 epitopes and confirm previous work demonstrating a close correlation between the lectin activity of LAM-1 (and MEL-14) and the HEV binding ability of leukocytes (Yednock et al., J. Cell. Biol. 104713, 1987; Yednock et al., J. Cell. Biol. 104:725, 1987; Stoolman et al., J. Cell. Biol. 99:1535, 1984; Rosen et al., J. Immunol. 142:1895, 1989; Stoolman et al., J. Clin. Invest. 84:1196, 1989; Stoolman et al., Blood 70:1842, 1987).

Activation of lymphocytes through CD2 or CD3, or activation of neutrophils with TNF-α or GM-CSF, induces a rapid and transient increase in the affinity of LAM-1 for its ligand. An interesting and unexpected result of the current studies was that binding of the anti-LAM1-1 and -5 mAb resulted in an identical increase in PPME binding by LAM-1 (Fig. 10). Crossblocking studies indicated that these mAb bind to the same or overlapping regions, distinct from those defined by the anti-LAM1-3 and -4 mAb. Interestingly, domain mapping studies demonstrated that these epitopes are in, or require the presence of, the LAM-1 EGF-like domain. However, only the anti-LAM1-1 mAb inhibited lymphocyte binding to HEV. The anti-LAM1-15 mAb, which blocks the

suggesting that the ligand for both murine and human lymphocytes is well conserved through recent mammalian evolution (Stoolman et al., Blood 70:1842, 1987; Stamper et al., J. Exp. Med. 144:828, 1976; Butcher et al., J. Immunol. 134:2989, 1979; Butcher et al., Nature 280:496, 1979; Wu et al., J. Cell. Biol. 107:1845). The demonstration that dog, tamarin and rabbit lymphocytes bind PPME in a calcium-dependent fashion (Fig. 12), in combination with the observation that dog and rhesus monkey lymphocytes utilized LAM-1 to bind to rodent HEV, confirms and extends this observation. These findings also indicate that the homing receptor expressed on leukocytes from these species involves a C-type lectin activity (Drickamer, J. Biol. Chem. 263:9557, 1988). Consistent with this, each mammal species (excluding rodents) tested expressed one or more of the LAM-1 epitopes (Table 11). The LAM1-3 epitope was the most broadly conserved among the animal species examined, further suggesting a critical function for this region in leukocyte migration. The reactivity of each mAb with numerous animal species also indicated that while several mAb were reactive with functionally identical regions of human LAM-1, most mAb identified unique epitopes. It is likely that subtle amino acid changes in LAM-1 between species accounts for the differences between reactivities of the anti-LAM-1 mAb. Thus these findings provide a mechanistic explanation for the conservation of receptor function, in that the regions of the molecule critical for adhesion were best conserved. Obtaining the primary amino acid sequences from LAM-1 cDNAs isolated from each species in the future will also be informative, but interpretation of sequence information in regards to ligand binding sites and functional epitopes of the molecule will require the exact knowledge of the three dimensional structure of LAM-1.

on lymphocytes is 74,000 and that f neutrophils i 90,000-100,000 [6,9,12].

The specific adhesion of some tumor cells to the capillary endothelium and the existence of organ specific metastasis suggest that interactions between tumor cells and normal tissues influence tumor localization [13-15]. Although the molecules that mediate these events in malignant cells have not been completely described, many cell surface molecules involved in the adhesion and migration of normal leukocytes may be involved in the dissemination of hematopoietic malignant cells [1-3]. The mLHR has been implicated in the dissemination of lymphomas [14-16], and a calcium-dependent phosphomannosyl-binding site on human malignant lymphoblastoid cell lines mediates peripheral LN HEV binding [17]. In the invention described herein, the structure, function and regulation of LAM-1 expression was examined on normal lymphocytes and compared to LAM-1 of malignant leukocytes.

The LAM-1 molecule is a member of a new family of cellular adhesion/homing molecules that contain a lectin-like domain at their amino-terminal end followed by an epidermal growth factor-like domain and short consensus repeat units like those found in C3/C4 binding proteins. In J. Exp. Med., 170: 123-133 (1989) [4] and co-pending Application Serial No. 07/313,109, T.F. Tedder et al. report the isolation and chromosomal localization of cDNAs encoding the novel cell surfac mol cul LAM-1. In Eur. J. Immun l., 20: 1351-1355 (1990), T.F. Tedd r t al. reported that human antigen-sp cific memory T c lls xpress th LAM-1 necessary for lymphocyt recirculation. In J. Bi logical Chemistry,

plays a key role in determining the characteristi s and magnitude of local immune responses [5].

The present invention relates to the production of a new antibody to LAM-1. The new monoclonal antibody, anti-LAM1-3, is useful in radioisotope or immunofluorescent assays for the detection of LAM-1. For example, identifying species which have or do not have LAM-1. The antibody is further useful for separating cells expressing LAM-1 from cells not expressing LAM-1 or visa versa. Furthermore, this monoclonal antibody also completely blocks leukocyte attachment to HEV or endothelium.

Neutrophil-mediated inflammation is involved in a number of human clinical manifestations, including the adult respiratory distress syndrome, multi-organ failure and reperfusion injury. One way of inhibiting this type of inflammatory response would be to block competitively the adhesive interactions between neutrophils and the endothelium adjacent to the inflamed region. Anti-LAM1-3 reacts with LAM-1 on many animal species, but does not bind the mLHR. Anti-LAM1-3 blocks completely lymphocytic traffic to lymph nodes and extravasation of neutrophils from blood to inflammatory sites. The administration of soluble forms of anti-LAM1-3 could be clinically effective for the inhibition of neutrophil-mediated inflammation. Anti-LAM1-3 also blocks lymphocyte adhesion to human HEV and activated endothelium. Therefore, it is likely that the use of anti-LAM1-3 will block lymphocyte entry into sites of inflammation or tissue injury. Such activity will be useful for pr v nting kidn y or other organ transplant rej ction which is m diat d by lymphocytes.

STATEMENT OF DEPOSIT

A hybrid cell line which produces the anti-LAM-1 monoclonal antibody anti-LAM1-3 embodying this invention was been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 on June 12, 1991 and is assigned A.T.C.C. Deposit No. HB 10771.

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protein (GMP-140, PADGEM, CD62) which is expressed on activated platelets and endothelial cells, and the human endothelial leukocyte adhesion molecule-1 (ELAM-1) expressed on activated endothelial cells. The name "selectins" has been suggested for this family because of the presence of the lectin domain and their role in selective cell trafficking.

LN = lymph node

PMA = phorbol 12-myristate 13-acetate

PKC = protein kinase C

LAM-1 = leukocyte adhesion molecule-1

CLL = chronic lymphocytic leukemia

NHL = non-Hodgkin's lymphoma

PPME = poly-phosphomonoester from the yeast HANSENULA cell wall

AML = adult myelogenous leukemia

CML = chronic myelogenous leukemia

PBMC = peripheral blood mononuclear cells

BM = bone marrow

RPMI Medium = commercial product available from Gibco, Walkersville, Maryland.

FSC = follicular small cleaved cell lymphoma

CSF = colony stimulating factor

DSC = diffuse small cleaved cell lymphoma

FITC = fluorescein isothiocyanate

LPS = lipopolysaccharide

kb = kilobase

shedding is similar to that of mLHR [33-34]. This is consistant with prior observations that the cell surface expression of LAM-1 is rapidly down-regulated upon activation [5,6,11]. LAM-1 removal from the cell surface may thus be necessary for the detachment of leukocytes from the endothelium so as to allow for their subsequent migration into tissues.

LAM-1 was most frequently expressed by CLL cells among the various hematologic malignancies studied (Table 1, Fig. 5). These results extend previous studies of LAM-1 expression to TQ1 and Leu-8 using CLL and NHL cells [35-37]. Since the expression of LAM-1 was somewhat restricted among hematologic malignancies, the expression, or absence of expression, may have a major impact on the trafficking of leukemic cells and the dissemination of NHL. Immunoprecipitation of LAM-1 from CLL cells showed that it resembled the Mr 74,000 isoform of the glycoprotein expressed by normal lymphocytes (Fig. 6). In addition, LAM-1 expressed by malignant cells was functional since LAM-1 on normal lymphocytes and CLL cells were both able to bind HEV and PPME (Table 2, Fig. 7). Both HEV and PPME binding was mediated by LAM-1 since the new monoclonal antibody, anti-LAM1-3, was able to completely block all HEV and PPME binding.

research group using lymphoblastoid cell lines [17]. CD44 constitutes a broadly distributed family of glycoproteins expressed on virtually all hematopoietic cells, fibroblasts, epidermal, glial and melanocytic origin cells [21,38]. Although CD44 was initially regarded as the human homing receptor equivalent of the mLHR [28,29], it may be more generally involved in cell-cell or cell-matrix binding as a receptor for hyaluronate [39]. Previous studies have also suggested that CD44 is involved in the dissemination of NHL [40]. During the work resulting in the present invention, however, no clear relationship could be inferred from the results of CD44 expression alone.

LAM-1 is expressed on most neutrophils, monocytes, normal myeloid progenitor cells and early erythroid precursors in BM (bone marrow) [6]. The co-expression of this homing receptor and other adhesion molecules may control the physiological retention (homing) of these cells in BM. The homing of intravenously transplanted hematopoietic stem cells is mediated by a recognition system with galactosyl and mannosyl specificities [41] which might also mimic the LAM-1 ligand [42]. In this regard, it is noted that AML and CML cells were found to lack expression of LAM-1. Unlike the situation with lymphoid tumors, this is in sharp distinction with the high level expression of LAM-1 on normal myeloid cells. The absence of LAM-1 expression on most AML and CML cells might favor the passage of these cells into the bloodstream. Although overnight culture of CML cells did not result in the expression of LAM-1 on th c ll surfac , the overall lack of LAM-1 expr ssion by these c lls indicat s that further investigations of the regulation of LAM-1 by l ukemic myeloid cells is warranted.

th Human Protection Committee f the Dana-Farb r Cancer Institute. Tumor type was classified according to conventional morphological, cytological and immunophenotype criteria. Tumor cell lineage was determined by analysis of antigens (Ag) including surface and cytoplasmic immunoglobulin (Ig), HLA-DR Ag, CD1, CD2, CD3, CD4, CD5, CD6, CD8, CD9, CD10, CD11b, CD13, CD14, CD19, CD20 and CD33. Cells were examined immediately after isolation or were immediately cryopreserved and kept frozen in liquid nitrogen until used. The frequency of malignant cells was always greater than 90% in every sample examined.

Antibodies.

The anti-LAM-1 monoclonal antibodies anti-LAM1-1 and anti-LAM1-2 and the monoclonal antibody anti-TQ1 have been previously described [5,8]. The anti-LAM1-3 antibody (IgG1) of the claimed invention was generated by the fusion of NS-1 myeloma cells with spleen cells from Balb/c mice that were repeatedly immunized with cells of the mouse pre-B cell line 300.19 transfected with a LAM-1 cDNA as described [5]. The antibodies used in these studies included: 2F12 (CD11a) and 10F12 (CD18) [18] which were gifts from J. Ritz (Dana-Farber Cancer Inst., Boston MA); TS2/9 (CD58, anti-LFA-3) [19] and RR 1/1 (CD54, anti-ICAM-1) [20] which were gifts from T.A. Springer (Center for Blood Research, Boston, MA); 515 (CD44) [21] a gift from G.S. Kansas (Dana-Farber Cancer Inst.); and 904 (CD11b) [22].

Immunofluorescence analysis.

Indirect immunofluoresc nce analysis was p rform d on viabl cells isolated by Ficoll-Hypaque density gradient

Immunoprecipitation analysis

Cells were washed twice, resuspended in RPMI 1640 medium (Sigma, St. Louis, MO) at a concentration of 30 x 10^6 cells/ml) and treated for 40 minutes at room temperature with neuraminidase (0.1 U/ml, Calbiochem, La Jolla, CA) and then labelled by lactoperoxidase-catalyzed iodination. After washing, the cells were lysed in buffer containing 1% (v/v) NP-40 as described [27]. Cell lysates were precleared for 2 hours using 3 µl of murine ascites fluid (isotype matched antibody) and 25 µl of a 50% (v/v) suspension of Gammabind-G Agarose (Genex, Gaithersburg, MD). Cell lysates were precleared again overnight. Half of the precleared lysate was then incubated with 3 µl of anti-TQ1 ascites fluid, 3 µl of anti-LAM1-1 ascites fluid, and 50 µl of Gammabind-G with constant rotation at 4°C for 18 hours. The other half of the lysate was treated similarly using 3 µl of isotype-matched murine ascites fluid. Immunoprecipitates were washed and analyzed by SDS-PAGE. Molecular weights (Mr) were determined using standard molecular weight markers (BRL, Bethesda, MD).

In experiments designed to study LAM-1 shedding, LAM-1 was immunoprecipitated as described above from the supernatant fluid and the pellet of PBMC that had been cultured for 60 minutes at 37°C in RPMI 1640 medium alone or in RPMI medium containing PMA (100 ng/ml, Sigma, St. Louis, MO). In addition, expression of LAM-1 was assessed after incubation of the cells with PMA (10 nM for 30 minutes) following the prior culture of the cells with sodium azide (Sigma) or the protein kinase inhibitors, 1-(5-Isoquinolinyl-sulfonyl)-2-methylpiperazine (H-7, Calbiochem) and staurosporine (Sigma) for 30 minutes at 37°C.

myelog nous l ukemia; CML, chr nic myelogenou leukemia). The relative fluorescence staining intensity of the malignant cells is indicated where the positive population could be identified as a distinguishable peak from background fluorescence staining: ±, where a shoulder of positively stained cells was evident, +, where a separate peak of positive cells was identified with weak fluorescence; ++, a definite separate peak of fluorescence positive cells of moderate fluorescence; +++, a peak of fluorescence positive cells of the same intensity as normal blood lymphocytes. The tissue source of all malignant cells is also indicated.

Figure 6.

Anaylsis of LAM-1 immunoprecipitated from B-CLL cells. Detergent lysates of surface iodinated cells (45 x 10^6) were immunoprecipitated with the anti-TQ1 and anti-LAM1-1 antibodies (LAM-1) or an unreactive isotype-matched antibody control (Cont.). Immunoprecipitated materials wer divided and analyzed under non-reducing and reducing conditions on a 12% SDS polyacrylamide gel followed by autoradiography. Molecular weights (kDa) were determined by the migration of known protein standards.

Figure 7.

Normal human lymphocytes and CLL cells are capable of binding PPME through LAM-1. Cells were examined for LAM-1 expression by indirect immunofluorescence analysis after treatment with the anti-LAM1-3 monoclonal antibody (dark line) or with an unreactiv isotype matched antibody (thin line). Cells were also reacted with FITC-conjugated PPME aft r treatment with th anti-LAM1-3 antibody (thin line) or an unreactive control antibody (dark lin). The

iodination since LAM-1 may be more readily immunoprecipitated after the removal of sialic acid residues. Treatment of cells with PMA resulted in a dramatic loss of immunoprecipitable LAM-1 from the cell surface with a concomitant increase in the level of LAM-1 precipitated from the supernatant fluid. Incubation of cells in medium without PMA also resulted in a small amount of LAM-1 being found in the supernatant fluid (Fig. 4). The molecular weight of LAM-1 precipitated from the supernatant fluid was slightly smaller (by about 5 kDa) than the species of LAM-1 found on the cell surface. Interestingly, the residual LAM-1 found on the cell surface of PMA-treated cells was most similar in molecular weight to that of the LAM-1 found in the supernatant fluid. The quantititive recovery of labeled LAM-1 from the supernatant fluid, in comparison to the amount immunoprecipitated from solubilized cells, demonstrates that a major portion of LAM-1 is shed from the cell surface and not internalized following PMA exposure.

Expression of adhesion molecules by malignant leukocytes.

The expression of LAM-1 and other cell surface molecules known to be involved in lymphocyte adhesion and migration was examined on malignant leukocytes from 118 patients by indirect immunofluorescence analysis. LAM-1 expression was most frequently demonstrated on CLL cells and among lymphomas classified as follicular (FSC) and diffuse small cleaved cell lymphoma (DSC) (Table 3). On the other hand, most acute myeloblastic leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelocytic leukemia (CML),

appearanc of LAM-1 on the cell surfaces or a significant increase in the frequency of LAM-1 expression in any case.

Structure of LAM-1 expressed by malignant cells.

Anti-LAM-1 antibodies were used to immunoprecipitate LAM-1 from CLL cells. LAM-1 migrated with a Mr of 68,000 under non-reducing conditions and at 73,000 after reduction (FIG. 6), similar to LAM-1 immunoprecipitated from normal lymphocytes (FIG. 4). Therefore, it appears that normal and malignant lymphocytes express the same cell-surface LAM-1 protein.

LAM-1 receptor function.

The relationship between LAM-1 expression and the ability of cells to bind to human peripheral LN HEV was examined using cells from normal circulating blood, three LAM-1 positive CLLs and one LAM-1 negative CLL. Cells were assessed for their ability to bind HEV of human peripheral LN using the frozen section assay of Stamper and Woodruff [25]. The LAM-1+ cells bound to HEV at levels which corresponded to the amount of LAM-1 expressed on their cell surface, while the LAM-1 CLL cells did not bind (Table 2). In contrast, CD44 expression was quite high on all of the cell samples examined and did not correlate with HEV adhesion. Additional studies examined the ability of anti-LAM-1 monoclonal antibody to block HEV binding. A new antibody, anti-LAM1-3, was abl to sp cifically block 92 to 95% of normal lymphocyt and LAM-1+ CLL cell binding (cells from Table 2) to rat p ripheral LN HEV. In contrast, the binding f a different antibody, anti-LAM1-10, reactive with a différent

LAM-1+ CLL cell samples tested further, PMA exposure led to the complete loss of LAM-1 expression after 180 to 360 minutes of culture with PMA with similar kinetics to those of RAJI cells treated simultaneously. These experiments were also carried out using cryo-preserved blood lymphocytes and RAJI cells as controls, with no appreciable affect on the ability of the cells to modulate LAM-1 expression after PMA exposure.

The down-modulation of LAM-1 expression was also investigated in LAM-1 cDNA transfected cells. The erythroleukemia cell line, K562, and the mouse pre-B cell line, 300.19, were transfected with LAM-1 cDNA as described [5], generating cells that express relatively high levels of cell surface LAM-1 (Fig. 8). In contrast to what was observed with RAJI cells and the majority of CLL cells, 90 minutes exposure of these cells to PMA induced an almost complete loss of LAM-1 from the cell surface.

The role of PKC in LAM-1 shedding was further assessed by culturing normal blood lymphocytes with protein kinase prior to their exposure to PMA. Treatment of cells with both H-7 [31] and staurosporine [32] inhibited shedding, albeit at different optimal molar concentrations (Table 1). In contrast, pretreatment of lymphocytes with sodium azide did not inhibit down-modulation of cell surface receptor. However, the shedding process required on-going metabolism since PMA treatment at 4°C did not induce detectable LAM-1 shedding (data not shown). Thus, PKC may regulate cell surface receptor expression through direct phosphorylation of LAM-1 which may signal for cleavage or through kinase regulation of protease activity.

two patients with AML, one with ALL and on with CML) w re incubated overnight at 37°C in RPMI 1640 medium containing 10% FCS. Contrary to the results obtained under the same conditions with normal spleen cells [5], an increase in LAM-1 expression was not detected on the malignant cells after culture, suggesting that LAM-1 was not expressed constitutively on these cells.

Table 5, below, lists a number of the properties of the monoclonal antibodies anti-LAM1-1, -2 and -3. Lines 1, 2, and 3 give the name of the antibody, the isotype and the differences in staining intensity. These properties are not necesarily indicative of differences in epitope recognition.

Functional studies of the antibodies are given in lines 4-6. These results demonstrate that different parts of the LAM-1 molecule are recognized by the different monoclonal antibodies. Lymph nodes contain structures called high endothelial venules (HEV) which are utilized by lymphocytes to enter the lymph nodes (the site of immune responses) from the blood stream. Emigration of lymphocytes into the node has been shown to be mediated by adhesion molecules which allow the cells to stick to and then traverse the venule. This process has been studied by incubating isolated lymphocytes with lymph node tissue sections. When the sections are incubated with lymphocytes alone, the cells will adhere to HEV, and the number of adherent cells can be counted. Various monoclonal antibodies, including the LAM-1 antibodies, have be n used to block this binding, Lin 4 giv s the results of such studies for LAM1-1, -2 and -3.

The polysaccharide PPME mimics the natural ligand for the LAM-1 mol cul . Since PPME can b directly fluores-

LAM1-3 strongly blocks it. Speci s cross-reactivity giv s further indications of the diferences which exist between the antibodies and the epitopes that they identify.

Line 11 gives the results of the domain mapping regarding the monoclonal antibodies. The LAM-1 molecule contains three domains which are:

(a) a lectin-like domain (L);

(b) an epidermal growth factor-like domain (EGF); and

(c) a domain of short consesus repeats (SCR).

In order to determine which domain was recognized by each antibody, cDNAs were constructed which contained the information coding for:

(1) the whole LAM-1 molecule;

(2) the L, EGF and SCR domains from the LAM-1 molecule;

(3) the L domain from the LAM-1 plus EGF and SCR domains from the CD62 molecule of the same family of proteins;

(4) the L plus EGF domains from LAM-1 (SCR from CD62; and

(5) the L plus SCR domains from LAM-1 (EGF from CD62).

These cDNAs were transfected into cells which then produced the corresponding proteins. The pattern of reactivity of the various monoclonal antibodies was then determined as shown in Table 6, and the domain necessary for monoclonal antibody reactivity was assigned. For example, anti-LAM1-3 bound to cells expressing all the domains described with m dium to very strong strength. Anti-LAM1-1, however, did not bind to c lls which contained LAM-1 (L + SCR) or LAM-1 (L) alone. The pitope which is recognized by anti-LAM1-1 must, ther fore, be compos d of a sit within the EGF,

The wide conservation of the LAM-1 epitopes expressed throughout recent mammalian evolution, in combination with the functional studies demonstrating a molecular component similar to LAM-1 in function and specificity, underscores the critical role of LAM-1 in the regulation of leukocyte migration in multiple animal species. Thus, LAM-1 may be the most functionally and structurally conserved leukocyte adhesion molecule found in mammals, considering that many do not function reciprocally between man and mouse. Whether the evolutionary basis for this high level of conservation is fundamental to the function of the receptor or results from the unique nature of a carbohydrate-based ligand with limited potential for divergence will have to be determined after identification and characterization of the true ligand(s).

Use

As leukocyte migration and infiltration into areas of tissue damage or injury or tissue transplant can cause or increase pathology, agents that impede these processes can be used for therapeutic treatment. Specifically, leukocyte-mediated inflammation is involved in a number of human clinical manifestations, including the adult respiratory distress syndrome, multi-organ failure and reperfusion injury. One way of inhibiting this type of inflammatory response would be to block competitively the adhesive interactions between leukocytes and the endothelium adjacent to the inflamed region. As LAM-1 mediates the migration and adhesion of blood leukocytes, treatment of a patient in shock, e.g., from a serious injury, with an antagonist to c ll surface LAM-1 function (such as the monoclonal antibodies of the invention) can result in the reduction of

Table 1. LAM-1 Shedding Is Blocked By Protein Kinase Inhibitors

		Without PMA[a] Treatment		With PMA Treatment	
Cells with		% Positive	MCF No.	% Positive	MCF No.
Medium		60	115	13	47
Stauro					
porine	1mM	57	91	54	95
	500μM	72	95	49	91
	100μM	51	88	51	92
	50μM	47	92	58	100
H-7	1mM	52	94	53	92
	500μM	67	102	32	80
	100μM	58	109	23	91
	50μM	61	106	11	61
NaN_3	1μM	59	113	20	50

a = The percentage of cells reactive with the anti-LAM1-1 monoclonal antibody was determined by indirect immunofluorescence analysis. The relative intensity of staining of the positive cells is indicated based on the mean fluorescence channel number (MFC No.) obtained with FACS analysis (256 channels, on a 3-decade log scale. Cells treated with an unreactiv monoclonal antibody had 3% positiv cells with a MFC no. of 40.

Table 3

EXPRESSION OF ADHESION MOLECULES BY MALIGNANT LEUKOCYTES

	No of cases expressing antigen / number examined[a] (mean % among positive cases)						
Diagnosis	LAM-1	CD44	CD11a	CD11b	CD18	CD54	CD58
Pre-B-ALL							
CD10+	4/15 (32)	10/12 (67)	1/8 (46)	0/15	1/12 (40)	4/13 (33)	6/13 (69)
CD10-	0/6	5/5 (80)	0/6	0/5	0/5	0/5	2/5 (55)
T-ALL	2/10 (31)	8/9 (67)	3/8 (59)	0/2	5/10 (49)	4/10 (46)	1/10 (62)
B-CLL	16/27 (55)	16/18 (76)	3/14 (45)	0/21	3/18 (32)	1/18 (31)	1/18 (43)
B-lymphoma							
FSC	6/12 (32)	9/11 (59)	8/9 (56)	0/12	9/11 (38)	5/11 (50)	2/11 (49)
DSC	2/4 (41)	4/4 (70)	1/4 (90)	0/3	1/4 (90)	3/4 (40)	1/4 (77)
DLC	1/6 (27)	6/6 (59)	5/6 (55)	0/6	4/4 (49)	3/6 (54)	2/6 (53)
Burkitt's	0/4	2/4 (52)	0/4	0/4	0/4	1/4 (72)	0/4
M. Myeloma	0/3	2/2 (84)	3/3 (53)		2/2 (37)	0/3	1/3 (52)
AML	2/19 (44)	16/16 (79)	6/11 (50)	5/19 (42)	11/16 (41)	1/16 (47)	9/16 (61)
CML	0/12	10/10 (61)	5/10 (43)	2/12 (48)	5/10 (39)	0/10	4/10 (70)

[a]Cases were considered positive for the Ag being examined if > 25% of the cells were positive.

2. HEV binding was assessed using 12-μm freshly cut, frozen rat lymph node sections. The number of lymphocytes bound to HEV was counted on coded slides. Values are means ± standard deviation (s.d.) of four experiments, and the differences between control antibody-treated cells and anti-CD2 and anti-CD3 treated cells were significant.

3. $P<0.05$ using the paired Student's t-test.

4. $P<0.01$ using the paired Student's t-test

1 = Fluorescence intensity, human lymphocyte and peripheral mononuclear cells. Fluorescence intensity of staining in indirect immunofluorescence assays is given on a 4-(+) scale where (-) indicates no specific reactivity and (++++) indicates the highest level of activity.

2 = Monoclonal antibody blocking, High Endothelial Venule binding.

3 = Monoclonal antibody blocking, phosphomannan monoester fragments (PPME).

4 = PPME blocking monoclonal antibody.

5 = Ability to block binding of antibody.

6 = Domain mapped.

Abbreviations

Bind = Binding

B = Blocks

wB = weakly Blocks

E = Enhances

ND = Not Done

L = Lectin

EGF = Epidermal Growth Factor-like

SCR = Short Consensus Repeats

N = No effect

Table V

Properties of the anti-LAM-1 mAb used in this study

Name	mAb Isotype	Staining Intensity[a] Lympho.	Neutro.	HEV[b] Binding	Effect[c] on PPME
TQ1	G1	++	++	N	B
LAM1-1	G1	+++	+++	B	E
LAM1-2	M	++	++	wB	B
LAM1-3	G1	++++	++++	B	B
LAM1-4	G1	+++	+++	B	B
LAM1-5	G1	+++	+++	N	E
LAM1-6	G1	+	+	B	N
LAM1-7	G1	++	++	N	N
LAM1-8	G1	+++	+++	N	N
LAM1-9	G1	+++	+++	N	N
LAM1-10	G1	+++	+++	N	N
LAM1-11	G1	+++	+++	N	N
LAM1-12	G1	+++	+++	N	N
LAM1-13	M	+	+	N	N
LAM1-14	G1	+++	++	N	N
LAM1-15	G1	++	++	N	N
LAM1-16	M	+	+	N	N

[a] The reactivity of lymphocytes and neutrophils with the anti-LAM-1 mAb were determined by indirect immunofluorescence analysis with the relative intensity of fluorescence staining indicated on a - (no reactivity) to ++++ (highest reactivity) scale as in Fig. 1. Results are representative of those obtained in at least three experiments.

[b] The ability of each mAb to inhibit lymphocyte binding to rat HEV was assessed as in Table 8. B = Blocks binding, E = Enhances binding, N = No significant or detectable effect on binding, w = weak effect. Results are representative of those obtained in at least two xperiments.

[c] The ability of each mAb to inhibit PPME binding to human lymphocytes was as in Fig. 2. Results are representative of those obtained in at least two experiments.

Table 9

Cross-Blocking Experiments with Anti-LAM-1 mAb[a]

	Ability of the test mAb to block the binding of labeled:				
Test mAb:	anti-TQ1	Leu-8	LAM1-1	LAM1-3	LAM1-5
TQ1	+++	-	-	+++	ND
LAM1-1	-	++	+++	-	+++
LAM1-2	+++	-	-	+++	ND
LAM1-3	+++	+++	-	+++	-
LAM1-4	+++	+++	-	+++	-
LAM1-5	++	++	+++	-	+++
LAM1-6	+++	-	+	-	-
LAM1-7	+++	++	++	-	++
LAM1-8	-	++	++	-	++
LAM1-9	-	+++	+++	-	+++
LAM1-10	-	+++	+++	-	+++
LAM1-11	-	+++	+++	-	+++
LAM1-12	-	+++	+++	-	+++
LAM1-14	-	-	++	-	-
LAM1-15	-	++	+++	-	+++

[a] Values represent the relative ability of the test mAb to block the binding of the indicated FITC-labeled antibodies: -, did not inhibit; +, partial inhibition; ++, significant but incomplete inhibition; +++, complete inhibition of mAb binding. These results are representative of those obtained in three experiments.

Table 11

CONSERVATION OF LAM-1 EPITOPES AMONG DIFFERENT ANIMAL SPECIES

mAb	HUMAN[a]	RHESUS[a]	TAMARIN[a]	COW[a]	RABBIT[a,b]	SHEEP[a]	DOG[a]	CAT[a]	PIG[b]	GOAT[b]	RAT[a,b]	GUINEA PIG[b]	CHICKEN[b]
TQ1	+++	-	-	ND	+++	-	-	-	ND	ND	-	ND	ND
LAM1-1	+++	+++	+++	ND	-	-	-	-	ND	ND	-	ND	ND
LAM1-2	+++	-	++	ND	+ +	-	-	-	ND	ND	-	ND	ND
LAM1-3	++++	+	-	+++	++++	+++	+++	+++	+	+++	-	-	-
LAM1-4	+++	-	++	+ + +	+ + +	-	-	-	+ + +	ND	-	-	-
LAM1-5	+++	+++	+++	+ + +	+	+ + +	-	-	-	+ + +	-	-	-
LAM1-6	+++	+	+	-	+	-	-	-	-	ND	-	-	-
LAM1-7	+++	++	++	+ +	+ +	-	-	-	-	ND	-	-	-
LAM1-8	+++	++	+++	+++	-	++	-	-	-	ND	-	-	-
LAM1-9	+++	++	++	+++	-	+++	-	-	-	ND	-	-	-
LAM1-10	+++	++	++	+++	-	-	-	-	-	ND	-	-	-
LAM1-11	+++	+++	+++	+++	-	-	-	-	-	ND	-	-	-
LAM1-12	+++	+++	++	++	-	ND	-	-	-	ND	-	-	-
LAM1-13	±	-	-	-	+	+	-	-	-	ND	-	-	-
LAM1-14	+++	+	+	++	++	-	-	+	-	ND	-	-	-
LAM1-15	+++	++	++	+++	-	+++	-	-	-	+++	-	-	-
LAM1-16	+	-	+	-	-	+	-	-	-	ND	-	+	-

[a] Reactivity and relative fluorescence intensity of staining was determined by flow cytometry analysis of blood leukocytes. The scale of fluorescence intensities is as in Fig. 1 with - indicating no significant reactivity. ND, not determined.

[b] Reactivity of staining was determined by immunohistology of thymus, lymph nodes and rabbit appendix.

leukocyte migration to a level manageable by the target endothelial cells.

In addition, agents developed to block receptor function can inhibit the metastasis and homing of malignant cells which express the LAM-1 receptor protein.

The therapeutic agents may be administered orally, parenterally, or topically by routine methods in pharmaceutically acceptable inert carrier substances. Optimal dosage and modes of administration can readily be determined by conventional protocols.

The normal regulation of the *lyam-1* gene (the name given to the human gene encoding LAM-1), as evidenced by the appearance and disappearance of the LAM-1 protein on the surface of a specific leukocyte subpopulation, can be monitored by use of the monoclonal antibodies of the invention, in order to test the effects of drugs or specific therapies that may alter gene expression.

What is claimed is:

7. The method of claim 5, wherein said monoclonal antibody is anti-LAM1-4 monoclonal antibody.

8. A method of detecting leukocyte activation in an animal, said method comprising

obtaining a fluid sample from said animal;

contacting said sample with the monoclonal antibody of claim 1; and

detecting formation of immune complexes formed by said monoclonal antibody, said immune complexes indicating the presence of LAM-1 shed from activated leukocytes.

9. The method of claim 8, wherein said monoclonal antibody is anti-LAM1-4 monoclonal antibody.

10. A method of blocking leukocyte interactions with endothelium in an animal, said method comprising administering an effective amount of the monoclonal antibody or fragment thereof of claim 1 to said animal.

11. The method of claim 10, wherein said monoclonal antibody is anti-LAM1-4 monoclonal antibody.

12. The method of claim 10, wherein said animal is a human.

13. The method of claim 10, wherein said animal is suffering from inflammation.

14. The method of claim 10, wherein said animal is suffering from an autoimmune response.

contacting said cells with a reagent comprising the monoclonal antibody of claim 18; and

determining which cells form an immune complex with said reagent.

23. A method of isolating cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with the monoclonal antibody of claim 18; and

separating those cells which have formed an immune complex with said monoclonal antibody, from those cells which have not.

24. The method of claim 22, wherein said monoclonal antibody is anti-LAM1-5 monoclonal antibody.

25. A method of detecting leukocyte activation in an animal, said method comprising

obtaining a fluid sample from said animal;

contacting said sample with the monoclonal antibody of claim 18; and

detecting formation of immune complexes formed by said monoclonal antibody, said immune complexes indicating the presence of LAM-1 shed from activated leukocytes.

26. The method of claim 25, wherein said monoclonal antibody is anti-LAM1-5 monoclonal antibody.

27. A method of blocking leukocyte interactions with endothelium in an animal, said method comprising

35. A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-6 monoclonal antibody.

36. The monoclonal antibody of claim 35, wherein said monoclonal antibody is anti-LAM1-6 monoclonal antibody.

37. A hybridoma cell which produces the monoclonal antibody of claim 35.

38. The hybridoma cell line deposited as ATTC #_____.

39. A method of identifying cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with a reagent comprising the monoclonal antibody of claim 35; and

determining which cells form an immune complex with said reagent.

40. A method of isolating cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with the monoclonal antibody of claim 35; and

separating those cells which have formed an immune complex with said monoclonal antibody, from those cells which have not.

41. The method of claim 39, wherein said monoclonal antibody is anti-LAM1-6 monoclonal antibody.

50. The method of claim 46, wherein said monoclonal antibody is a chimerized antibody having a variable region derived from anti-LAM1-6 monoclonal antibody and constant regions derived from a human antibody.

51. A method of determining the degree of expression of LAM-1 in a sample of leukocytes, said method comprising

contacting a sample of leukocytes with the antibody of claim 35, and

determining the level of immune complex formation in said sample, said level being indicative of the degree of expression of LAM-1 on said leukocytes.

52. A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-7 monoclonal antibody.

53. The monoclonal antibody of claim 52, wherein said monoclonal antibody is anti-LAM1-7 monoclonal antibody.

54. A hybridoma cell which produces the monoclonal antibody of claim 52.

55. The hybridoma cell line deposited as ATTC #_____.

56. A method of identifying cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with a reagent comprising the monoclonal antibody of claim 52; and

62. The method of claim 61, wherein said monoclonal antibody is anti-LAM1-7 monoclonal antibody.

63. The method of claim 61, wherein said animal is a human.

64. The method of claim 61, wherein said animal is suffering from inflammation.

65. The method of claim 61, wherein said animal is suffering from an autoimmune response.

66. The method of claim 61, wherein said animal is suffering from rejection of an organ or tissue transplant.

67. The method of claim 63, wherein said monoclonal antibody is a chimerized antibody having a variable region derived from anti-LAM1-7 monoclonal antibody and constant regions derived from a human antibody.

68. A method of determining the degree of expression of LAM-1 in a sample of leukocytes, said method comprising

contacting a sample of leukocytes with the antibody of claim 52, and

determining the level of immune complex formation in said sample, said level being indicative of the degree of expression of LAM-1 on said leukocytes.

69. A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-8 monoclonal antibody.

obtaining a fluid sample from said animal;

contacting said sample with the monoclonal antibody of claim 69; and

detecting formation of immune complexes formed by said monoclonal antibody, said immune complexes indicating the presence of LAM-1 shed from activated leukocytes.

77. The method of claim 76, wherein said monoclonal antibody is anti-LAM1-8 monoclonal antibody.

78. A method of blocking leukocyte interactions with endothelium in an animal, said method comprising administering an effective amount of the monoclonal antibody or fragment thereof of claim 69 to said animal.

79. The method of claim 78, wherein said monoclonal antibody is anti-LAM1-8 monoclonal antibody.

80. The method of claim 78, wherein said animal is a human.

81. The method of claim 78, wherein said animal is suffering from inflammation.

82. The method of claim 78, wherein said animal is suffering from an autoimmune response.

83. The method of claim 78, wherein said animal is suffering from rejection of an organ or tissue transplant.

84. The method of claim 80, wherein said monoclonal antibody is a chimerized antibody having a variable region

91. A method of isolating cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with the monoclonal antibody of claim 86; and

separating those cells which have formed an immune complex with said monoclonal antibody, from those cells which have not.

92. The method of claim 90, wherein said monoclonal antibody is anti-LAM1-9 monoclonal antibody.

93. A method of detecting leukocyte activation in an animal, said method comprising

obtaining a fluid sample from said animal;

contacting said sample with the monoclonal antibody of claim 86; and

detecting formation of immune complexes formed by said monoclonal antibody, said immune complexes indicating the presence of LAM-1 shed from activated leukocytes.

94. The method of claim 93, wherein said monoclonal antibody is anti-LAM1-9 monoclonal antibody.

95. A method of blocking leukocyte interactions with endothelium in an animal, said method comprising administering an effective amount of the monoclonal antibody or fragment thereof of claim 86 to said animal.

96. The method of claim 95, wherein said monoclonal antibody is anti-LAM1-9 monoclonal antibody.

105. A hybridoma cell which produces the monoclonal antibody of claim 103.

106. The hybridoma cell line deposited as ATTC #_____.

107. A method of identifying cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with a reagent comprising the monoclonal antibody of claim 103; and

determining which cells form an immune complex with said reagent.

108. A method of isolating cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with the monoclonal antibody of claim 103; and

separating those cells which have formed an immune complex with said monoclonal antibody, from those cells which have not.

109. The method of claim 107, wherein said monoclonal antibody is anti-LAM1-10 monoclonal antibody.

110. A method of detecting leukocyte activation in an animal, said method comprising

obtaining a fluid sample from said animal;

contacting said sample with the monoclonal antibody of claim 103; and

119. A method of determining the degree of expression of LAM-1 in a sample of leukocytes, said method comprising

contacting a sample of leukocytes with the antibody of claim 103, and

determining the level of immune complex formation in said sample, said level being indicative of the degree of expression of LAM-1 on said leukocytes.

120. A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-11 monoclonal antibody.

121. The monoclonal antibody of claim 120, wherein said monoclonal antibody is anti-LAM1-11 monoclonal antibody.

122. A hybridoma cell which produces the monoclonal antibody of claim 120.

123. The hybridoma cell line deposited as ATTC #_____.

124. A method of identifying cells expressing LAM-1, said method comprising

providing a sample of cells, at least some of which are suspected of expressing LAM-1;

contacting said cells with a reagent comprising the monoclonal antibody of claim 120; and

determining which cells form an immune complex with said reagent.

125. A method of isolating cells expressing LAM-1, said method comprising

132. The method of claim 129, wherein said animal is suffering from inflammation.

133. The method of claim 129, wherein said animal is suffering from an autoimmune response.

134. The method of claim 129, wherein said animal is suffering from rejection of an organ or tissue transplant.

135. The method of claim 131, wherein said monoclonal antibody is a chimerized antibody having a variable region derived from anti-LAM1-11 monoclonal antibody and constant regions derived from a human antibody.

136. A method of determining the degree of expression of LAM-1 in a sample of leukocytes, said method comprising

contacting a sample of leukocytes with the antibody of claim 120, and

determining the level of immune complex formation in said sample, said level being indicative of the degree of expression of LAM-1 on said leukocytes.

137. A monoclonal antibody which recognizes the LAM-1 epitope recognized by anti-LAM1-14 monoclonal antibody.

138. The monoclonal antibody of claim 137, wherein said monoclonal antibody is anti-LAM1-14 monoclonal antibody.

139. A hybridoma cell which produces the monoclonal antibody of claim 137.

detecting formation of immune complexes formed by said monoclonal antibody, said immune complexes indicating the presence of LAM-1 shed from activated leukocytes.

145. The method of claim 144, wherein said monoclonal antibody is anti-LAM1-14 monoclonal antibody.

146. A method of blocking leukocyte interactions with endothelium in an animal, said method comprising administering an effective amount of the monoclonal antibody or fragment thereof of claim 137 to said animal.

147. The method of claim 146, wherein said monoclonal antibody is anti-LAM1-14 monoclonal antibody.

148. The method of claim 146, wherein said animal is a human.

149. The method of claim 146, wherein said animal is suffering from inflammation.

150. The method of claim 146, wherein said animal is suffering from an autoimmune response.

151. The method of claim 146, wherein said animal is suffering from rejection of an organ or tissue transplant.

152. The method of claim 148, wherein said monoclonal antibody is a chimerized antibody having a variable region derived from anti-LAM1-14 monoclonal antibody and constant regions derived from a human antibody.

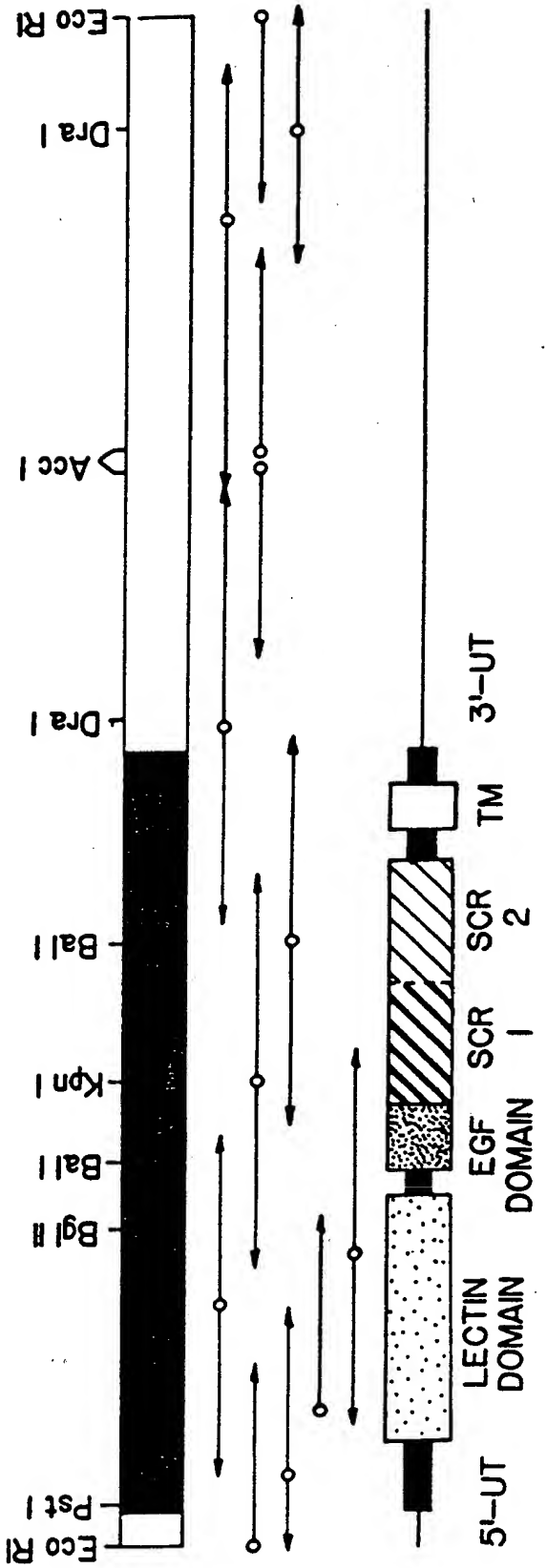

Eco RI
Dra I
Acc I
Dra I
Bal I
Kpn I
Bal I
Bgl II
Pst I
Eco RI
5'-UT
LECTIN DOMAIN
EGF DOMAIN
SCR 1
SCR 2
TM
3'-UT

FIG. IA

FIG. IB

```
                                                                1
                                                                M   G
gaattcCCTTT GGGCAAGGAC CTGAGACCCT TGTGCTAAGTCAAGAGGCTCA ATG GGC
                                10
 C   R   R   T   R   E   G   P   S   K   A   M
TGC AGA AGA ACT AGA GAA GGA CCA AGC AAA GCC ATG    94
                    20                              30
 I   F   P   W   K   C   Q   S   T   Q   R   D   L   W   N   I
ATA TTT CCA TGG AAA TGT CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC
                                40
 F   K   L   W   G   W   T   M   L   C   C   D
TTC AAG TTG TGG GGG TGG ACA ATG CTC TGT TGT GAT   168
                        50
 F   L   A   H   H   G   T   D   C ↓ W   T   Y   H   Y   S   E
TTC CTG GCA CAT CAT GGA ACC GAC TGC TGG ACT TAC CAT TAT TCT GAA
    60
 K   P   M   N   W   Q   R   A   R   R   F   C
AAA CCC ATG AAC TGG CAA AGG GCT AGA AGA TTC TGC   262
                            80
 R   D  [N   Y   T]  D   L   V   A   I   Q   N   K   A   E   I
CGA GAC [AAT TAC ACA] GAT TTA GTT GCC ATA CAA AAC AAG GCG GAA ATT
        90
 E   Y   L   E   K   T   L   P   F   S   R   S
GAG TAT CTG GAG AAG ACT CTG CCT TTC AGT CGT TCT   346
```

FIG. 2A

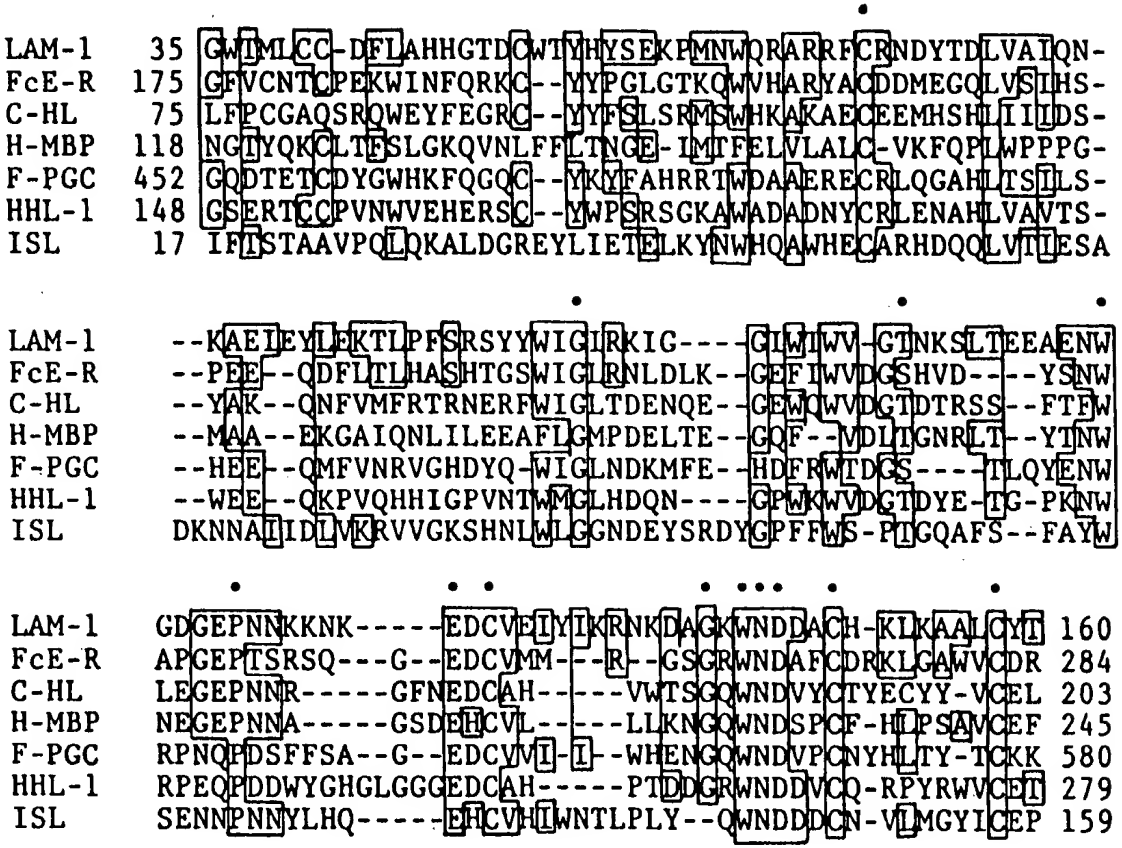
```
                                                                  110
    100                                                           I
 Y  Y   W   I   G   I   R   K   I   G   G   I   W   T   W
TAC TAC TGG ATA GGA ATC CGG AAG ATA GGA GGA ATA TGG ACG TGG
                            120
 V   G   T  [N   K   S]  L   T   E   E   A   E   N
GTG GGA ACC [AAC AAA TCT] CTC ACT GAA GAA GCA GAG AAC  430
                130                                          140
 W   G   D   G   E   P   N   N   K   K   N   K   E   D   C
TGG GGA GAT GGT GAG CCC AAC AAC AAG AAG AAC AAG GAG GAC TGC
                                    150
 V   E   I   Y   I   K   R   N   K   D   A   G   K
GTG GAG ATC TAT ATC AAG AGA AAC AAA GAT GCA GGC AAA  514
                        160
 W   N   D   D   A   C   H   K   L   K   A   A   L   C   Y
TGG AAC GAT GAC GCC TGC CAC AAA CTA AAG GCA GCC CTC TGT TAC
                                        180
170
 T   A   S   C   Q   P   W   S   C   S   G   H   G
ACA GCT TCT TGC CAG CCC TGG TCA TGC AGT GGC CAT GGA  598
                            190
 E   C   V   E   I   I   N  [N   Y   T]  C   N   C   D   V
GAA TGT GTA GAA ATC ATC AAT [AAT TAC ACC] TGC AAC TGT GAT GTG
                                                    210
            200
 G   Y   Y   G   P   Q   C   Q   F   V   I   Q   C
GGG TAC TAT GGG CCC CAG TGT CAG TTT GTG ATT CAG TGT  682
                                    220
 E   P   L   E   A   P   E   L   G   T   M   D   C   T   H
GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC ATG GAC TGT ACT CAC
                        230
 P   L   G   N   F   N   F   N   S   Q   C   A   F
CCT TTG GGA AAC TTC AAC TTC AAC TCA CAG TGT GCC TTC  766
                                            250
    240
 S   C   S   E   G   T  [N   L   T]  G   I   E   E   T   T
AGC TGC TCT GAA GGA ACA [AAC TTA ACT] GGG ATT GAA GAA ACC ACC
                        260
 C   E   P   F   G  [N   W   S]  S   P   E   P   T
TGT GAA CCA TTT GGA [AAC TGG TCA] TCT CCA GAA CCA ACC  850
                                                    280
            270
 C   Q   V   I   Q   C   E   P   L   S   A   P   D   L   G
TGT CAA GTG ATT CAG TGT GAG CCT CTA TCA GCA CCA GAT TTG GGG
                                        290
 I   M  [N   C   S]  H   P   L   A   S   F   S   F
ATC ATG [AAC TGT AGC] CAT CCC CTG GCC AGC TTC AGC TTT  934
                        300
 T   S   A   C   T   F   I   C   S   E   G   T   E   L   I
ACC TCT GCA TGT ACC TTC ATC TGC TCA GAA GGA ACT GAG TTA ATT
                                            320
310
 G   K   K   K   T   I   C   E   S   S   G   I   W
GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT GGA ATC TGG 1018
                                330
 S  [N   P   S]  P   I   C   Q   K   L   D   K   S   F   S
TCA [AAT CCT AGT] CCA ATA TGT CAA AAA TTG GAC AAA AGT TTC TCA
                                                    350
            340
 M   I   K   E   G   D   Y   N   P   L   F   I   P
ATG ATT AAG GAG GGT GAT TAT AAC CCC CTC TTC ATT CCA 1102
```

FIG. 2B

```
                                                    360
 V   A   V   M   V   T   A   F   S   G   L   A   F   I   I
GTG GCA GTC ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT
                        370
 W   L   A   R   R   L   K   K   G   K   K   S   K
TGG CTG GCA AGG AGA TTA AAA AAA GGC AAG AAA TCC AAG 1186
    380
 R   S   M   N   D   P   Y   *
AGA AGT ATG AAT GAC CCA TAT TAA ATCGCCCTTG GTGAAAGAAA

ATTCTTGGAA TACTAAAAAT CATGAGATCC TTTAAATCCT TCCATGAAAC 1280

GTTTTGTGTG GTGGCACCTC CTACGTCAAA CATGAAGTGT GTTTCCTTCA

GTGCATCTGG GAAGATTTCT ACCTGACCAA GAGTTCCTTC AGCTTCCATT 1380

TCACCCCTCA TTTATCCCTC AACCCCCAGC CCACAGGTCT TTATACAGCT

CAGCTTTTTC TCTTTTCTGA GGAGAAACAA ATAACACCAT AAAGGGAAAG 1480

GATTCATGTG GAATATAAAG ATGGCTGACT TTGCTCTTTC TTGACTCTTG

TTTTCAGTTT CAATTCAGTG CTGTACTTGA TGACAGACAC TTCTAAATGA 1580

AGTGCAAATT TGATACATAT GTGAATATGG ACTCAGTTTT CTTGCAGATC

AAATTTCGCG TCGTCTTCTG TATACGTCCA GGTACACTCT ATGAAGTCAA 1680

AAGTCTACGC TCTCCTTTCT TTCTAACTCC AGTGAAGTAA TGGGGTCCTG

CTCAAGTTGA AAGAGTCCTA TTTGCACTGT AGCCTCGCCG TCTGTGAATT 1780

GGACCATCCT ATTTAACTGG CTTCAGCCTC CCCACCTTCT TCAGCCACCT

CTCTTTTTCA GTTGGCTGAC TTCCACACCT AGCATCTCAT GAGTGCCAAG 1880

CAAAAGGAGA GAAGAGAGAA ATAGCCTCCG CTGTTTTTTA GTTTGGGGGT

TTTGCTGTTT CCTTTTATGA GACCCATTCC TATTTCTTAT AGTCAATGTT 1980

TCTTTTATCA CGATATTATT AGTAAGAAAA CATCACTGAA ATGCTAGCTG

CAACTGACAT CTCTTTGATG TCATATGGAA GAGTTAAAAC AGGTGGAGAA 2080

ATTCCTTGAT TCACAATGAA ATGCTCTCCT TTCCCCTGCC CCCAGACCTT

TTATCCACTT ACCTAGATTC TACATATTCT TTAAATTTCA TCTCAGGCCT 2180

CCCTCAACCC CACCACTTCT TTTATAACTA GTCCTTTACT AATCCAACCC

ATGATGAGCT CCTCTTCCTG GCTTCTTACT GAAAGGTTAC CCTGTAACAT 2280

GCAATTTTGC ATTTGAATAA AGCCTGCTTT TTAAGTGTTA AAAAgaattc 2330
```

FIG. 2C

FIG. 3A



SUBSTITUTE SHEET

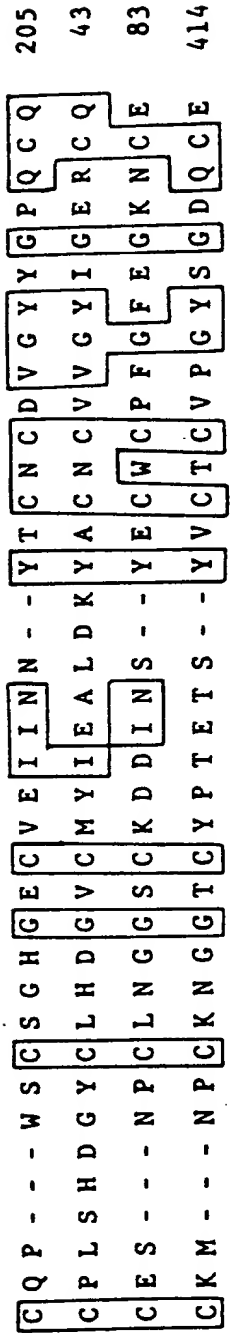

LAM-1 173 C Q P - - - W S C S G H G E C V E I I N N - - Y T C N C D V G Y Y G P Q C Q 205
EGF 6 C P L S H D G Y C L H D G V C M Y I E A L D K Y A C N C V V G Y I G E R C Q 43
F-IX 51 C E S - - - N P C L N G G S C K D D I N S - - Y E C W C P F G F E G K N C E 83
F-PGCP 382 C K M - - - N P C K N G G T C Y P T E T S - - Y V C T C V P G Y S G D Q C E 414

FIG. 3B

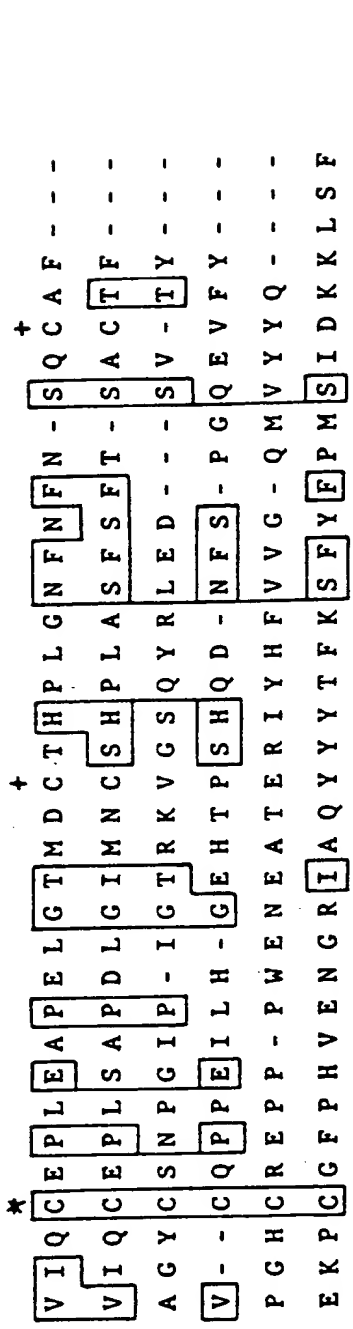

LAM-1 207 * V I Q C E P L E A P E L G T M D C T H P L G N F N F N - S Q C A F - - - + 268
LAM-1 269 V I Q C E P L S A P D L G I M N C S H P L A S F S F T - S A C T F - - - 330
Ba 137 A G Y C S N P G I P - I G T R K V G S Q Y R L E D - - - S V - T Y - - - 194
CR1 694 V - - C Q P E I L H - G E H T P S H Q D - N F S - P G Q E V F Y - - - 751
IL-2R 101 P G H C R E P P - P W E N E A T E R I Y H F V V G - Q M V Y Y Q - - - 164
F-XIII 1 E K P C G F P H V E N G R I A Q Y Y Y T F K S F Y F P M S I D K K L S F 68

FIG. 3C

LAM-1 * S C S E G T N - L - T G I E E - - - - T T C - - - - E P F G N W S S P E P T C Q *
LAM-1 I C S E G T E - L - I G K K K - T I C - - - - E S S G I W S N P S P I C Q
Ba H C S R G L T - L - R G S Q R - R T C - - - - Q E G G S W S G T E P S C Q
CR1 S C E P G Y D - L - R G A A S - L H C - - - - T P Q G D W S D E A P R C A
IL-2R - C V Q G Y R A L H R G P A E - S V C K M T H G K T R W T Q P Q L I C T
F-XIII F C L A G Y T T E S S R Q E E Q T T C T - T E - - G - W S - P E P R C F

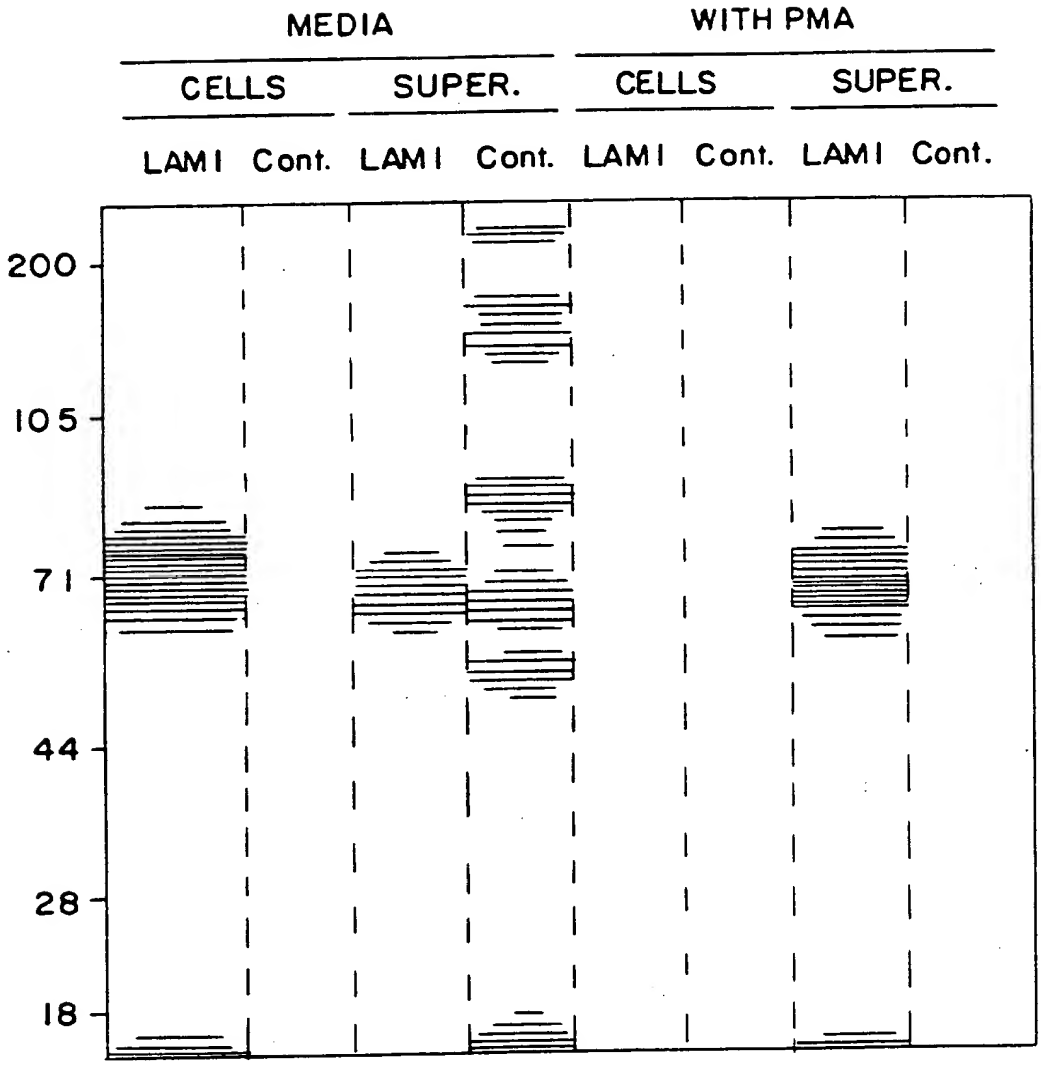

MEDIA
WITH PMA
CELLS
SUPER.
CELLS
SUPER.
LAMI
Cont.
LAMI
Cont.
LAMI
Cont.
LAMI
Cont.
200
105
71
44
28
18

FIG. 4

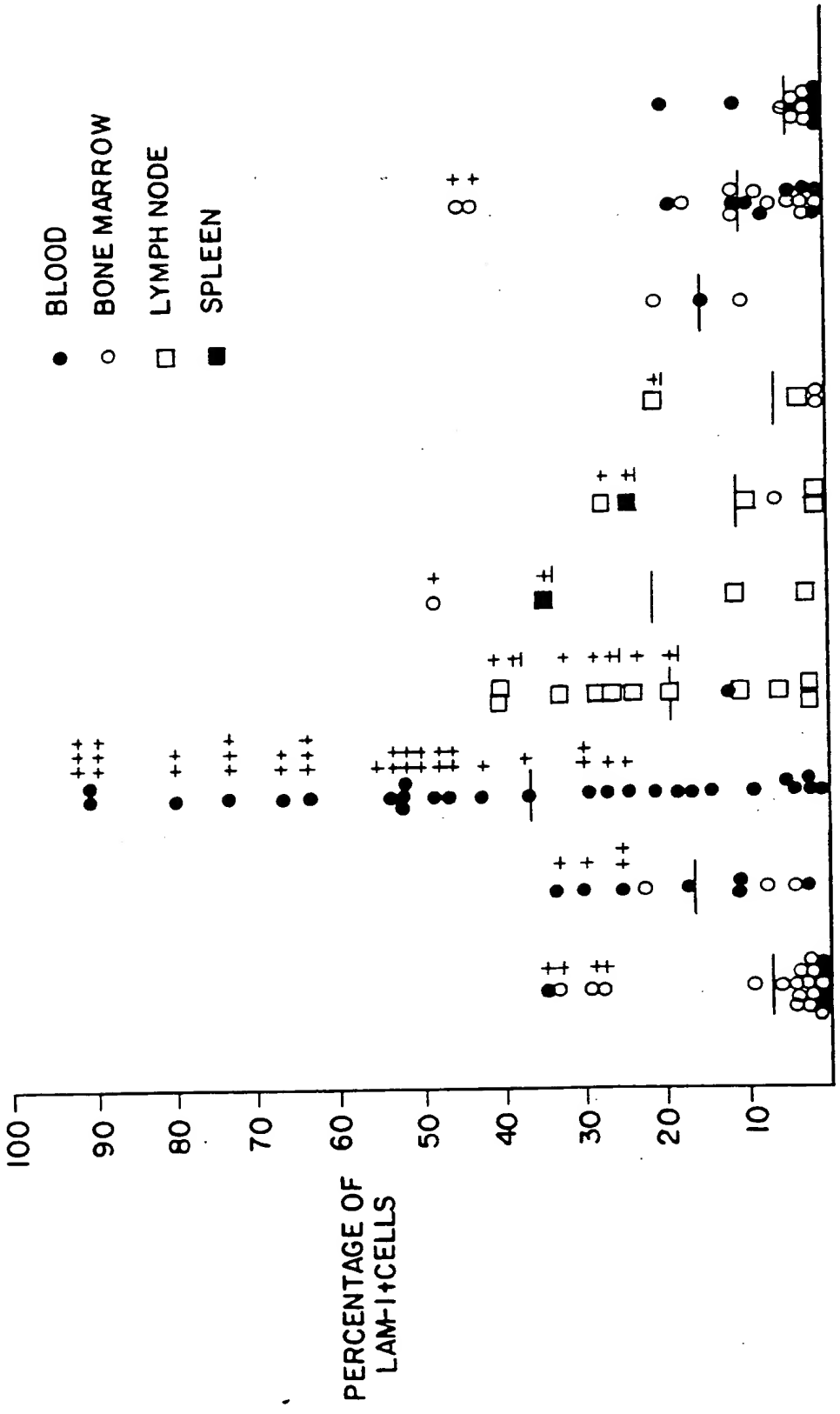

BLOOD
BONE MARROW
LYMPH NODE
SPLEEN
PERCENTAGE OF LAM-1+CELLS
100
90
80
70
60
50
40
30
20
10
Pre-B
T-ALL
B-CLL
FSC
DSC
DLC
Burk.
M.M.
AML
CML
DIAGNOSIS

FIG. 5

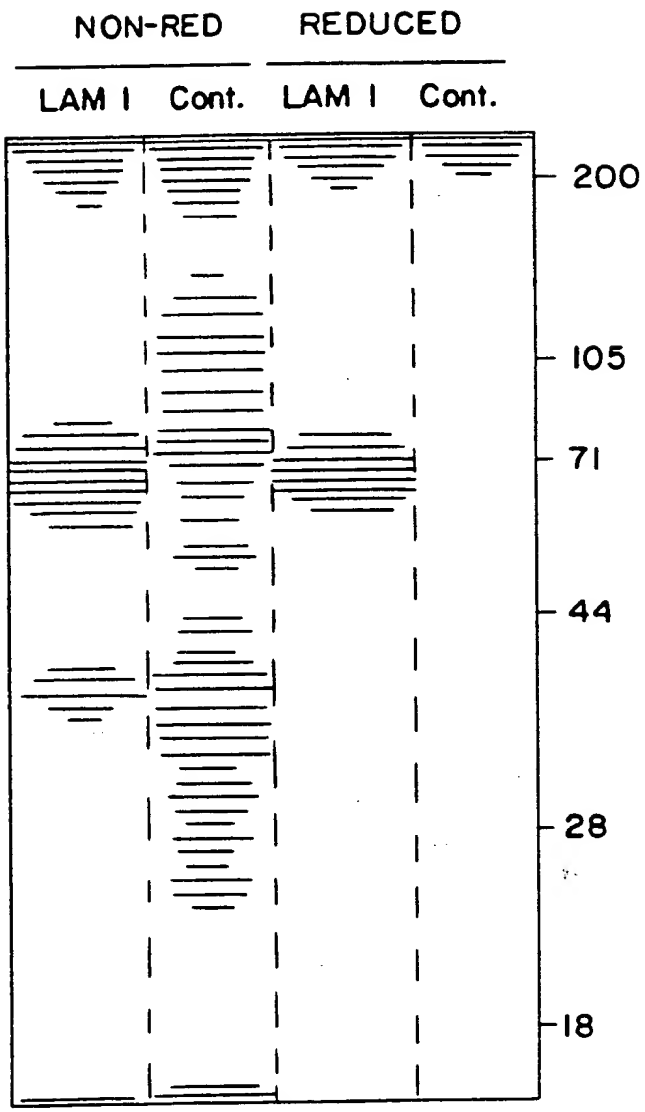


FIG. 6

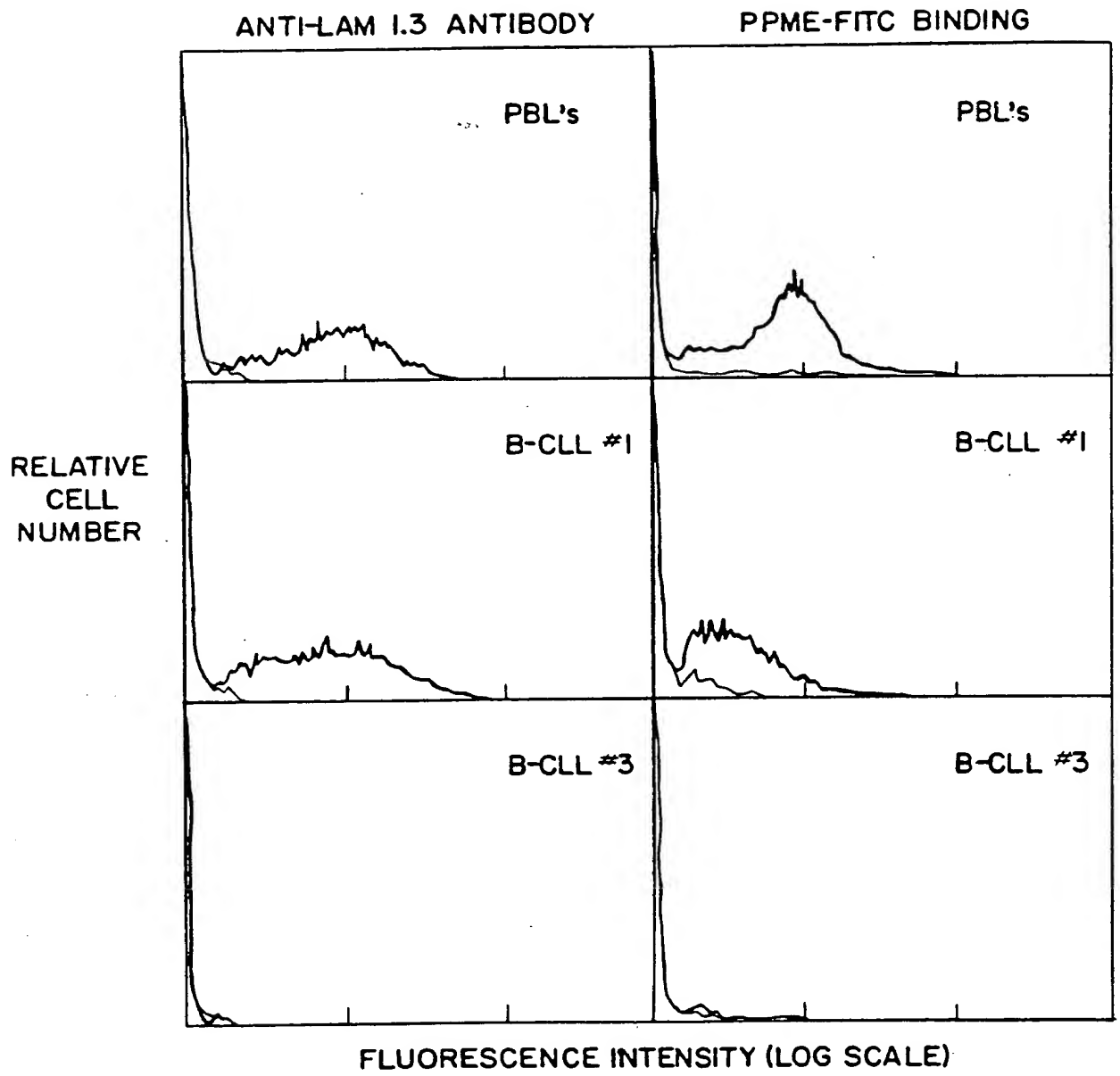

ANTI-LAM 1.3 ANTIBODY
PPME-FITC BINDING
PBL's
PBL's
B-CLL #1
B-CLL #1
B-CLL #3
B-CLL #3
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY (LOG SCALE)

FIG. 7

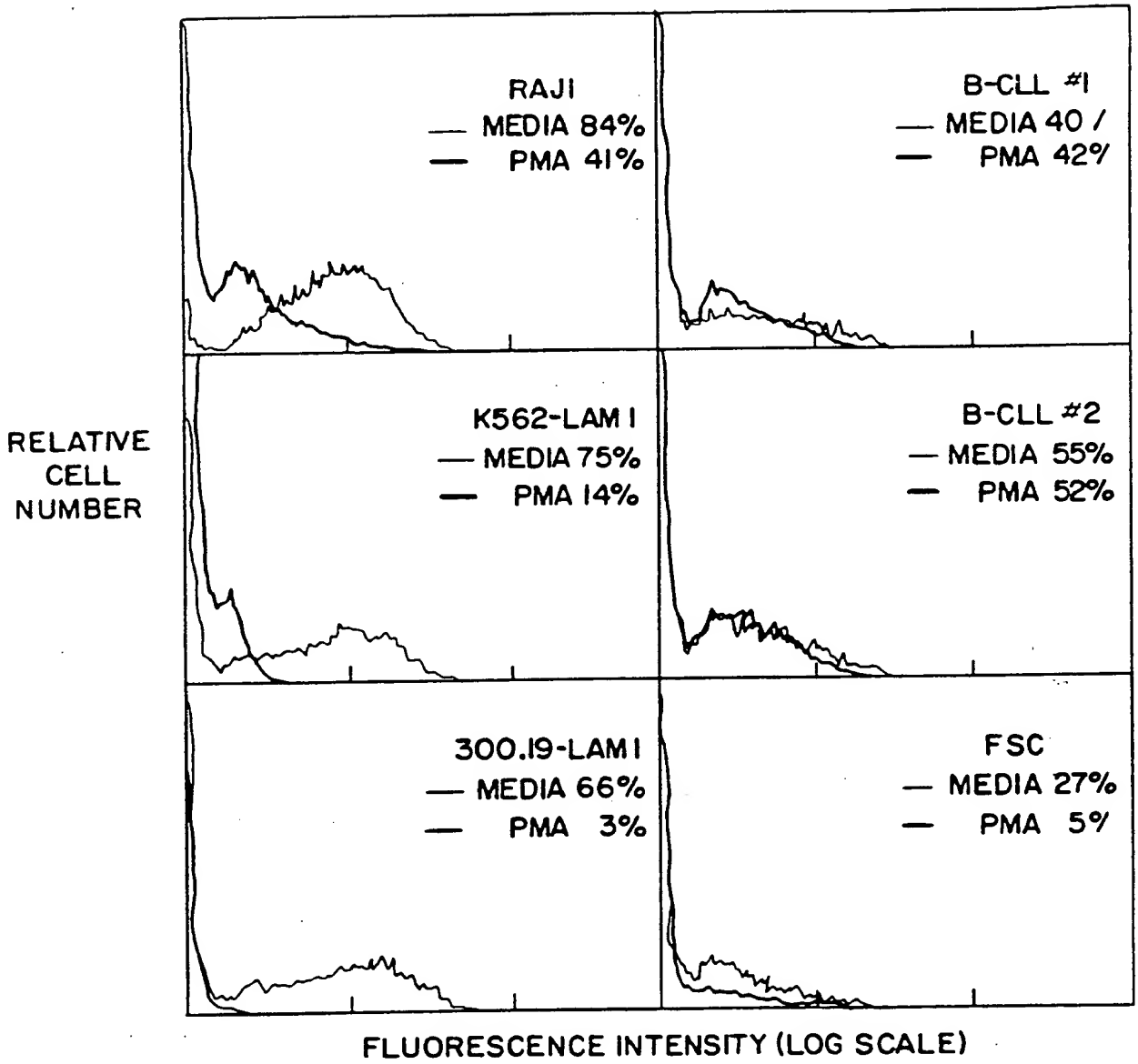

RAJI
— MEDIA 84%
— PMA 41%
B-CLL #1
— MEDIA 40 /
— PMA 42%
K562-LAM I
— MEDIA 75%
— PMA 14%
B-CLL #2
— MEDIA 55%
— PMA 52%
300.19-LAM I
— MEDIA 66%
— PMA 3%
FSC
— MEDIA 27%
— PMA 5%
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY (LOG SCALE)

FIG. 8

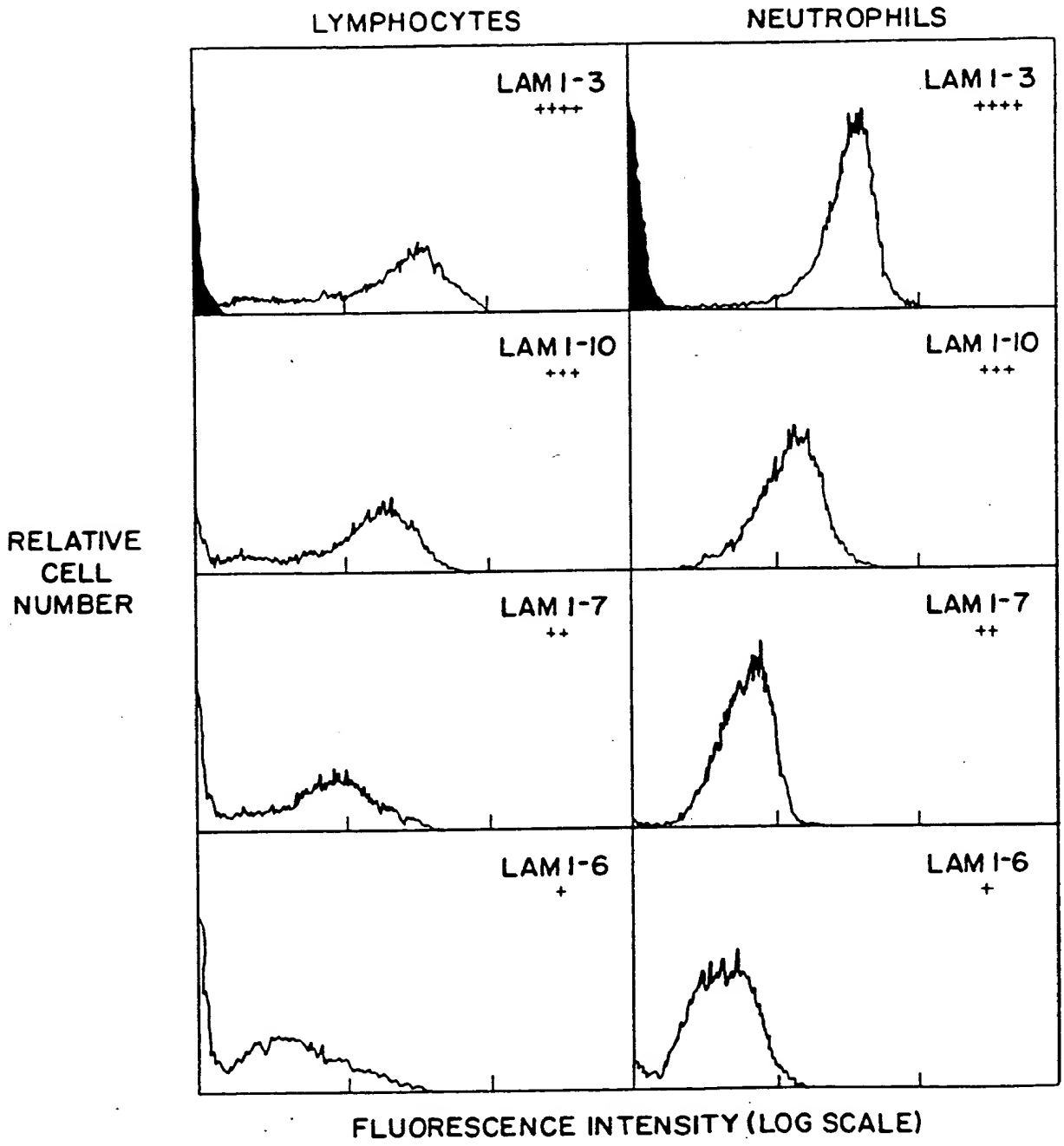

LYMPHOCYTES
NEUTROPHILS
LAM 1-3
++++
LAM 1-3
++++
LAM 1-10
+++
LAM 1-10
+++
LAM 1-7
++
LAM 1-7
++
LAM 1-6
+
LAM 1-6
+
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY (LOG SCALE)

FIG. 9

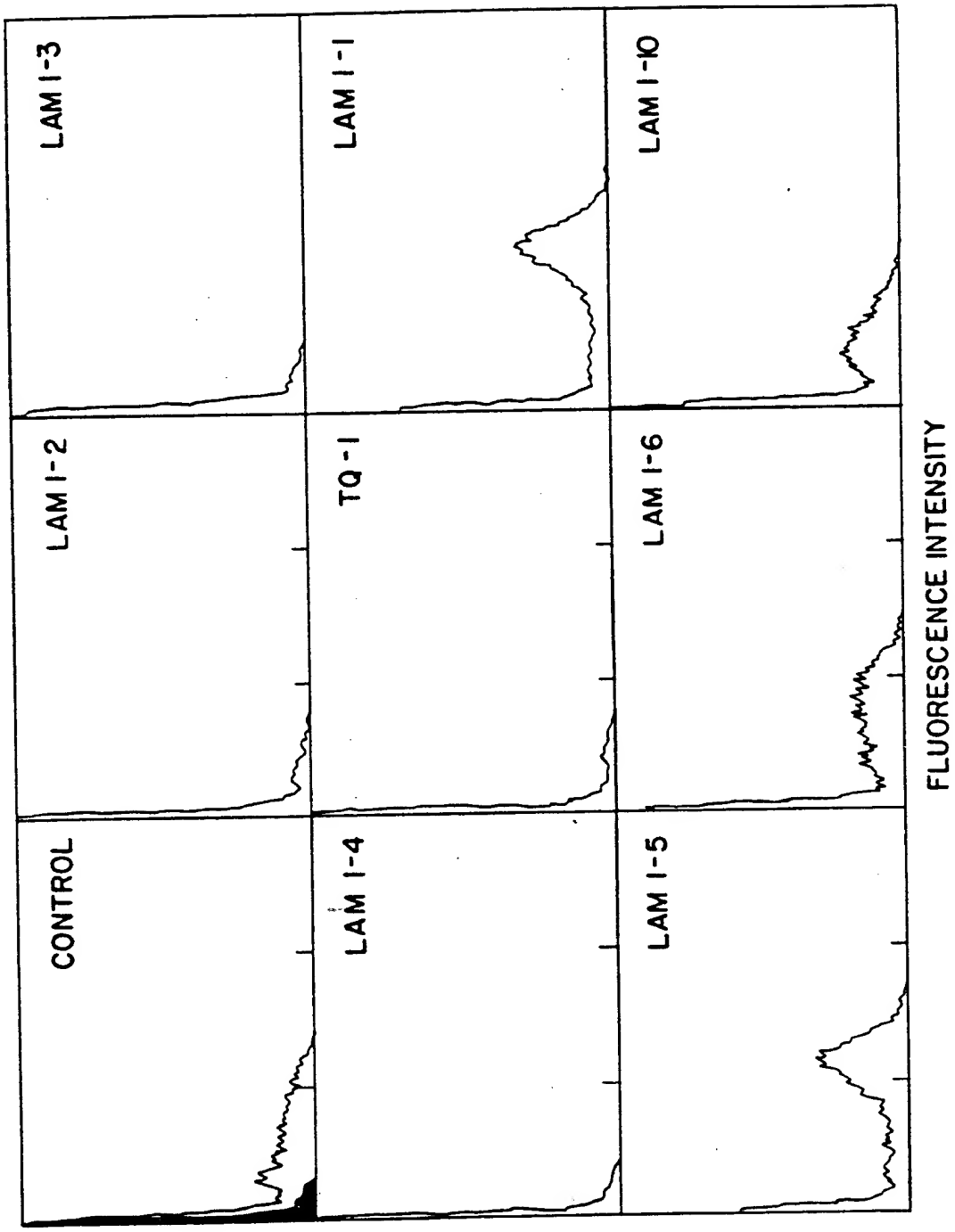

LAM I-3
LAM I-1
LAM I-10
LAM I-2
TQ-1
LAM I-6
CONTROL
LAM I-4
LAM I-5
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY

FIG. 10

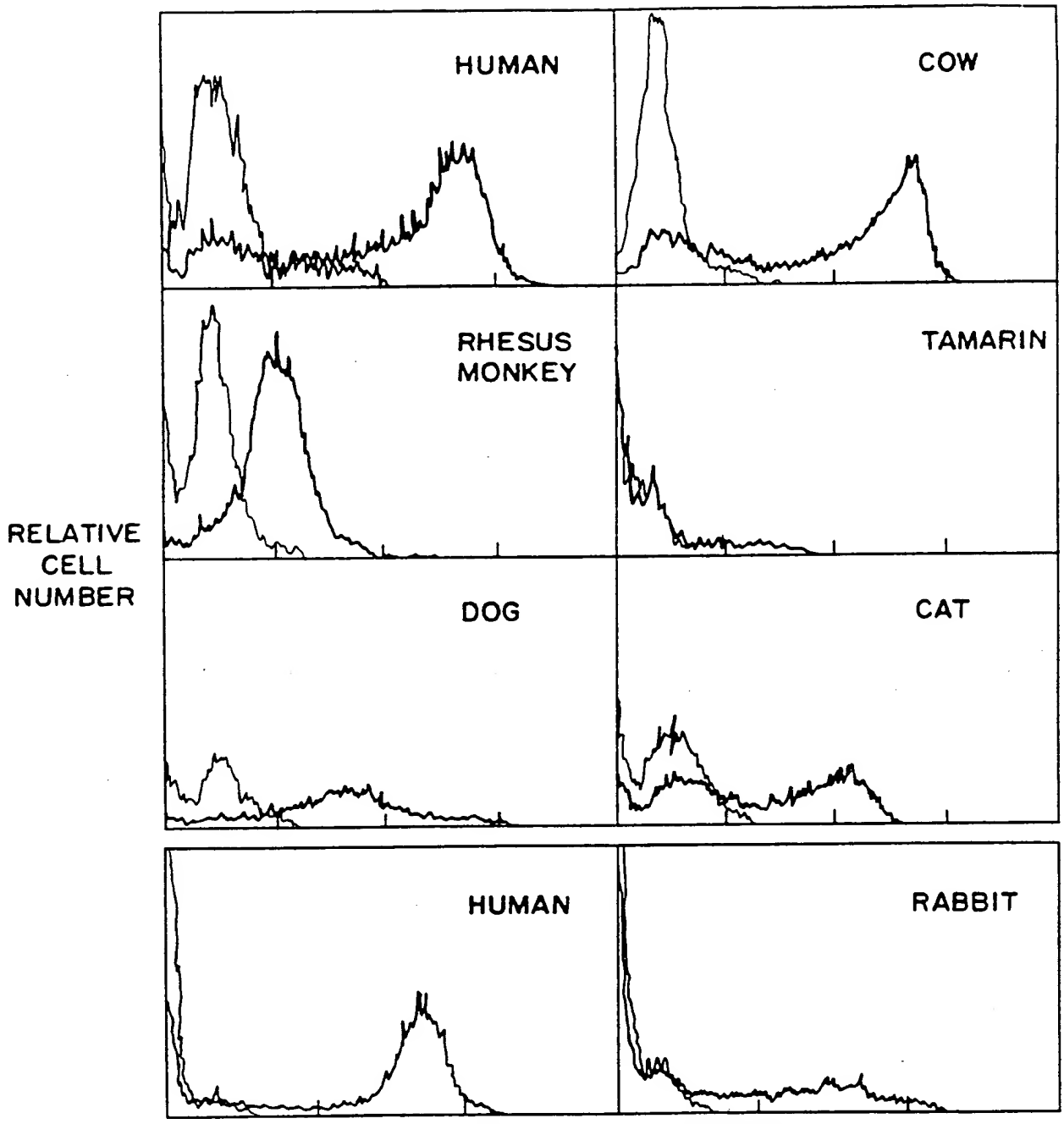

HUMAN
COW
RHESUS MONKEY
TAMARIN
DOG
CAT
HUMAN
RABBIT
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY

FIG. 11

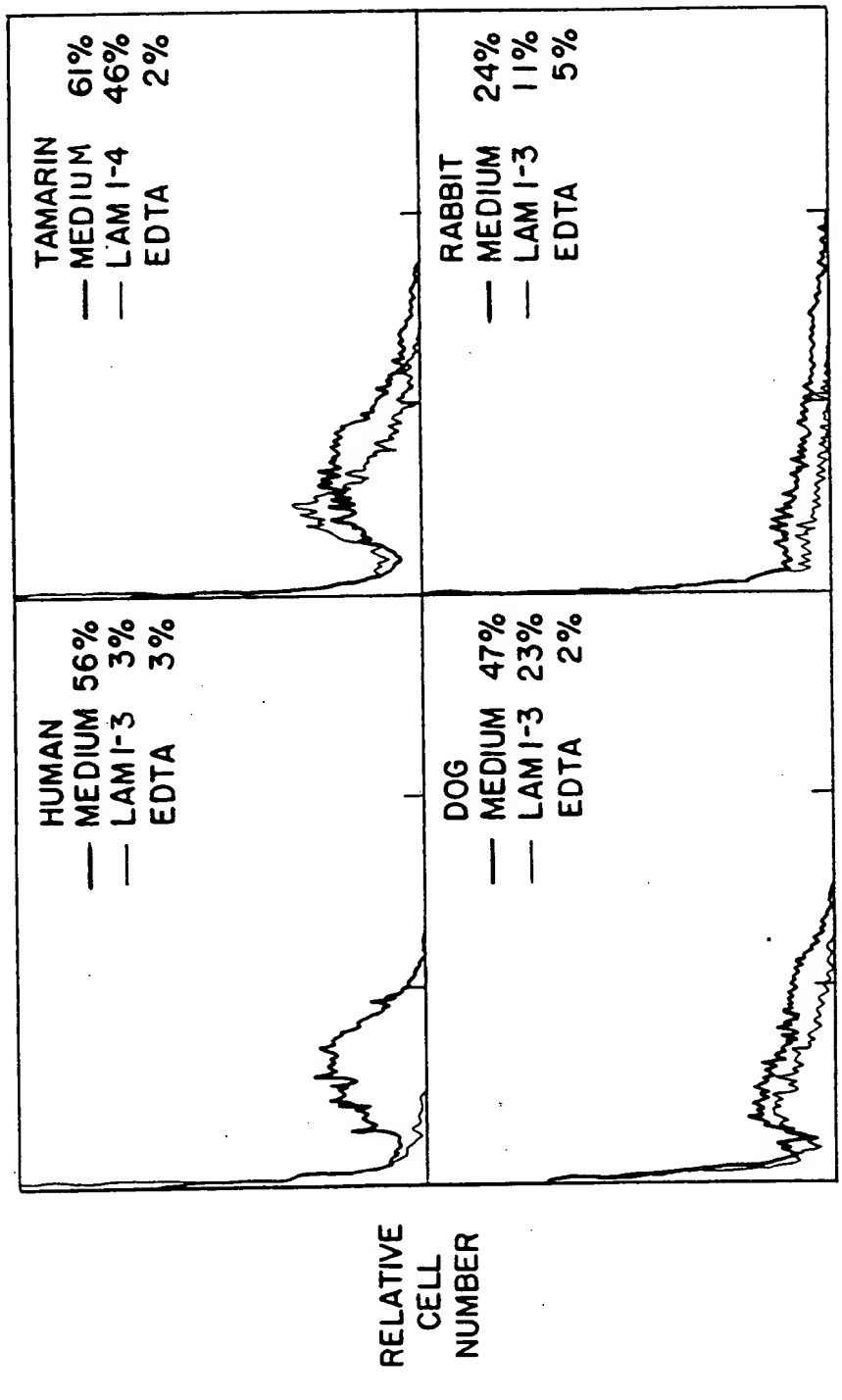

TAMARIN
MEDIUM 61%
LAM I-4 46%
EDTA 2%
RABBIT
MEDIUM 24%
LAM I-3 11%
EDTA 5%
HUMAN
MEDIUM 56%
LAM I-3 3%
EDTA 3%
DOG
MEDIUM 47%
LAM I-3 23%
EDTA 2%
RELATIVE CELL NUMBER
FLUORESCENCE INTENSITY

FIG. 12

FUNCTIONAL AND STRUCTURAL REGIONS OF LAM-1
IDENTIFIED BY MONOCLONAL ANTIBODIES

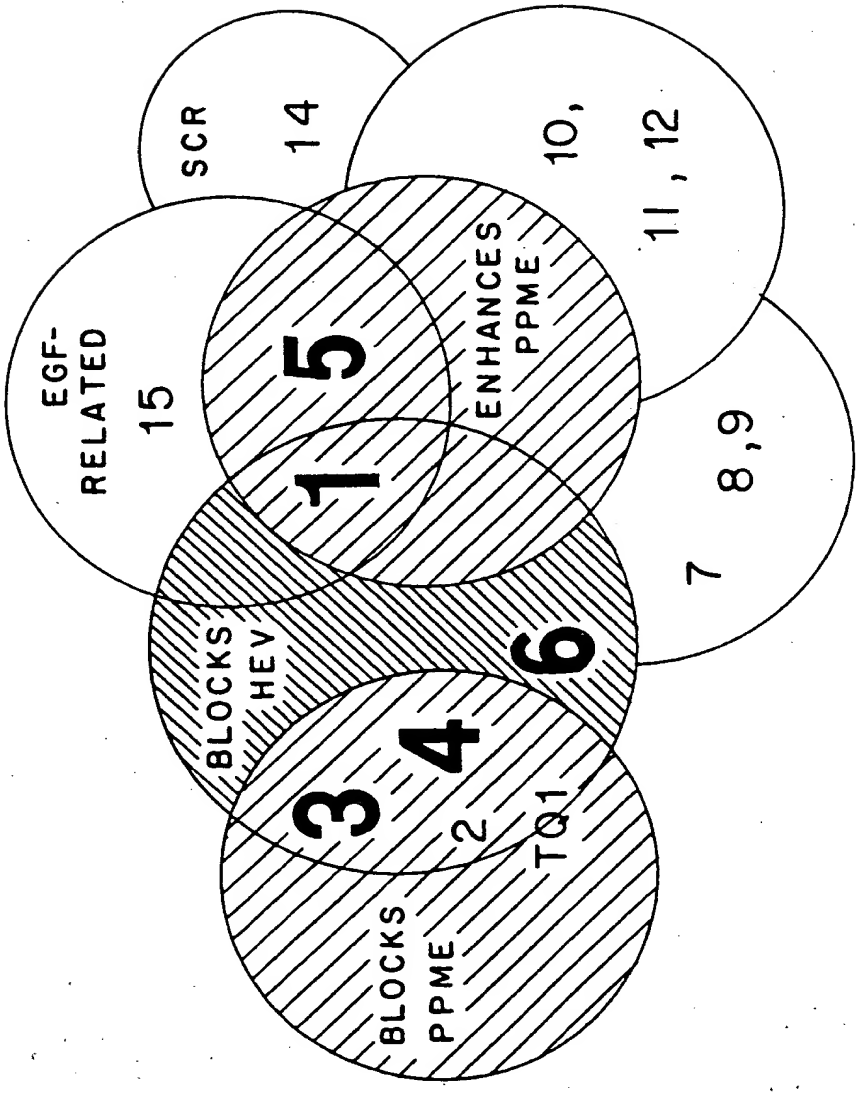


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06127

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/04, 39/00; C07K 13/00, 15/28; C12N 15/00, C12P 21/08
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.8; 435/70.21, 172.2; 530/387.1, 388.1, 388.22, 388.24, 388.85, 866, 867

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, MEDLINE, SCISEARCH, WPI, APS
SEARCH TERMS: LAM 1, ANTIBODY, TEDDER

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF IMMUNOLOGY, Volume 128, No. 1, issued 01 January 1982, E.L. Reinherz et al., "Heterogeneity of Human T4+ Inducer T cells Defined by a Monoclonal Antibody that Delineates Two Functional Subpopulations", pages 463-468, see entire document.	1-153
Y	PROC. NATL. ACAD. SCI. USA, Volume 87, issued March 1990, Kishimoto et al, "Identification of a Human Peripheral Lymph Node Homing Receptor: A Rapidly Down-Regulated Adhesion Molecule", pages 2244-2248, see entire document.	1-153
Y	JOURNAL OF CELL BIOLOGY, Volume 107, issued November 1988, N.W. Wu et al., "Evolutionary Conservation of Tissue-specific Lymphocyte-Endothelial Cell Recognition Mechanisms Involved in Lymphocyte Homing", pages 1845-1851, entire document.	1-153
Y	JOURNAL OF IMMUNOLOGY, Volume 144, issued 15 January 1990, T.F. Tedder, "Expression of the Human Leukocyte Adhesion Molecule, LAM1: Identity with the TQ1 and Leu-8 Differentiation Antigens", pages 532-540, see entire document.	1-153

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 SEPTEMBER 1992

Date of mailing of the international search report
16 SEP 1992

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06127

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF EXPERIMENTAL MEDICINE, Volume 170, issued 01 July 1989, T.F. Tedder et al., "Isolation and Chromosomal Localization of cDNAs Encoding a Novel Human Lymphocyte Cell Surface Molecule, LAM-1", pages 123-133, see entire document.	1-153
Y	JOURNAL OF CELL BIOLOGY, Volume 109, issued July 1989, B.R. Bowen et al., "Characterization of a Human Homologue of the Murine Peripheral Lymph Node Homing Receptor", pages 421-427, see entire document.	1-153
Y	USA 5,098,833 (Lasky et al.) 24 March 1992, see entire document.	1-153
Y	CELL, Volume 56, issued 24 March 1989, Stoolman, "Adhesion Molecules Controlling Lymphocyte Migration", pages 907-910, see entire document.	1-153
Y	CELL, Volume 62, issued 13 July 1990, Osborn, "Leukocyte Adhesion to Endothelium in Inflammation", pages 3-6, entire document.	1-153

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06127

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/85.8; 435/70.21, 172.2; 530/387.1, 388.1, 388.22, 388.24, 388.85, 866, 867